Abstract no.: W1.1 Effects of Nutrients on Biofilm Formation and Survivability of *H. pylori*

A. M. Hassanbhai, C. G. Ng and B. Ho
Department of Microbiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

*Helicobacter pylori* forms biofilm to overcome environmental stress. We show that *H. pylori* forms biofilm optimally in low nutrient and mild acidic conditions and that its biofilm-forming ability is strain-dependent. The study shows that growth is inversely proportional to biofilm formation as shown by enhanced growth rate in basal medium, whereas increased biofilm formation occurred in basal medium supplemented with cyclodextrin (with no known nutritional value). Interestingly, such biofilm and its exopolysaccharides were found to induce higher interleukin-8 production in AGS gastric epithelial cells, than its planktonic counterpart. Furthermore, the detection of 16S rRNA and the presence of viable *H. pylori* in drinking water and water biofilms as demonstrated by BacLight study coupled with the capacity of *H. pylori* to develop biofilm in milk indicate the probable association of *H. pylori* with biofilm in extragastric environment. Confocal and electron microscopy demonstrated that biofilm structures developed for survival are substrate-dependent with viable cells present several weeks post-biofilm formation. Taken together, it is postulated that biofilm may play an important role in the transmission of *H. pylori* in the extragastric environment.

Abstract no.: W1.2 Higher Gastric IL-10 Levels in *H. pylori*-Positive Children than Adults may not be Attributed to T<sub>reg</sub>-Foxp3 Cells

D. M. M. Queiroz,† G. A. Rocha,* F. F. Melo,* A. M. C. Rocha,* B. A. Chaves,* S. H. S. P. Pedroso,* L. P. F. Castro,* P. Bittencourt,‡ S. D. Carvalho† and R. Corrêa-Oliveira†
†Faculdade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil; ‡Hospital das Clínicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil; §FIOCRUZ/Centro de Pesquisas René Rachou, Belo Horizonte, Brazil

The immune response to *Helicobacter pylori* is more modulated in children than in adults, which has been attributed to increased T<sub>reg</sub> cells in children. Therefore, we evaluated the effect of *H. pylori* infection in children (*n* = 200) and compared infected children and adults (*n* = 100) with regard to gastric proinflammatory and T<sub>reg</sub> commitment ( interleukin (IL)-2, IL-10, and transforming growth factor (TGF)-β) cytokines, that were determined by ELISA (Biosource, Camarillo, CA, USA). T<sub>reg</sub>-Foxp3 cells were also assessed on the antral and corpus mucosa by immunoperoxidase. Although all cytokine mean levels were much higher in *H. pylori*-positive than in *H. pylori*-negative children (*p* < 10<sup>-3</sup> for all), the increase of proinflammatory cytokines of the innate immune arm (IL-6, IL-12p70, and tumor necrosis factor (TNF)-α) and of regulatory cytokine (IL-10) was more remarkable (fold increase > 33.3). Cytokine levels of the innate (IL-1α, IL-6, and TNF-α), modulatory (IL-10), and Th2 (IL-4) response (*p* < 10<sup>-5</sup> for all) were higher, and the levels of IL-1β (*p* < 10<sup>-3</sup>) IL-2 (*p* = .02), IL-17 (*p* = .003), IL-23 (*p* = .04), and interferon-γ (*p* = .01) were lower in children than in adults, but TGF-β (*p* = .23) did not differ. The T<sub>reg</sub>-Foxp3 cell median number was higher in *H. pylori*-positive than *H. pylori*-negative children (*p* = .001); however, it did not differ in the antrum (*p* = .63), but was decreased in the corpus (*p* = .03) of children compared to adults. Concluding, although IL-10 level is higher in infected children than in adults (6.1-fold), this may not be attributed to T<sub>reg</sub> cells, because their number and associated cytokines were similar in children and adults. Since innate immunity is strongly activated in children, we may propose dendritic cells/macrophages as an alternative IL-10 source.

Abstract no.: W1.3 Is There a Role for New *H. pylori* Virulence Markers in Pediatric Peptic Ulcer Disease?

M. Oleastro,* J. Cabral,† P. Ramalho,‡ P. Lemos,§ A. Santos* and A. Lopes‡
*Instituto Nacional de Saúde, Lisboa, Portugal; †Centro Hospitalar Lisboa Central, Lisboa, Portugal; ‡Hospital de Santa Maria, Lisboa, Portugal; §Hospital Fernando da Fonseca, Lisboa, Portugal

Aims: The development of *Helicobacter pylori*-associated peptic ulcer disease (PUD) is a relatively rare event in children; its occurrence may suggest a pathogenic role for the implicated strains. We aimed to compare the *H. pylori* genotype profile of strains isolated from children with PUD as compared to non-ulcer gastritis, using an extensive panel of *H. pylori* virulence markers.

Materials and Methods: One hundred and nine *H. pylori* strains were obtained from Portuguese children, during a 9-year period: 56 subjects had PUD (69% male, mean age 11.8 ± 3.3 years), of which 52 (92.9%) duodenal ulcer (DU) and 4 (7.1%) gastric ulcer; 53 subjects had non-ulcer gastritis (56.6% male, mean age 9.1 ± 3.6 years). Genotyping was performed by polymerase chain reaction and sequencing.

Results: The prevalence of *H. pylori* virulence genotypes was significantly higher in ulcer-associated strains than in non-ulcer gastritis strains (*p* < .01): cagA (79.5% vs 17%), vacA S1 (79.5% vs 18.9%), oipA (75.0% vs 32.1%), hopQ (59.1% vs 28.3%), and homB (81.8% vs 34%), with the exception of sabA, which was significantly associated with non-ulcer gastritis (22.6% vs 56.6%, *p* < .01).
Conclusions: H. pylori strains recovered from children with PUD, namely DU, showed a distinctive genotype virulence pattern, as compared to non-ulcer gastritis strains, suggesting a potential pathogenic role for new markers, such as homd. Thus, in some populations it is likely that the severity of H. pylori-associated disease in younger subjects may be closely related to the virulence of the strain, irrespective of the contribution of host and/or environmental factors, both playing a major role in the adult.

Abstract no.: W1.4
Is Apoptosis Activity a Marker of the Imbalances of Gastric Epithelial Proliferation in the H. pylori-Associated Gastritis in Children?

A. Durko, I. Planeta-Malecka, A. Kulig* and E. Czkwianianc‡
*Gastroenterology Department, Polish Mother’s Memorial Hospital, Research Institute, Lodz, Poland; ‡Pathology Department, Polish Mother’s Memorial Hospital, Research Institute, Lodz, Poland; †Department of Digestive Tract Diseases, Medical University, Lodz, Poland

Background: Increased cell proliferation and apoptosis induced by Helicobacter pylori infection plays a vital role in gastric carcinogenesis.

Aim: To assess H. pylori influence on gastritis and apoptotic activity in children.

Material and Methods: Seventy dyspeptic children (aged 8–17 years) underwent the upper gastrointestinal endoscopy with antral biopsies taken for pathology and apoptosis evaluation. Apoptosis was detected using the terminal deoxynucleotidyltransferase mediated dUTP nick-end labeling method (TUNEL). CagA was determined by PCR. Patients’ groups: Group I – H. pylori-associated gastritis; group II – gastritis, H. pylori negative; group III – normal stomach. H. pylori positive patients were followed up 6–8 weeks after eradication.

Results: In all the children with chronic gastritis (groups I and II) the inflammatory gastric lesions at endoscopy and pathology were seen. Much more severe inflammation and significantly higher degree of chronic and usually active gastritis according to the upgraded Sydney System in the group with H. pylori were observed (I vs II p < .001). Apoptosis index (AI) was significantly higher in group I (AI = 10.5 ± 7.12), (particularly in cagA-positive children) than in both remaining groups (p < .001). A strong positive correlation between a degree of chronic inflammation and expression of apoptosis was found. In the follow up of 23 children in which infection had been successfully eradicated, the decrease of inflammatory degree was found in both endoscopic and pathologic studies, with significant reduction of AI observed (p < .001), but not in subjects in whom eradication failed.

Table W1.5.1

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>AUC</th>
<th>Cut-off point</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>PLR</th>
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<tbody>
<tr>
<td>G-17</td>
<td>0.76</td>
<td>8 pmol/L</td>
<td>75%</td>
<td>69%</td>
<td>21%</td>
<td>96%</td>
<td>2.4</td>
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<tr>
<td>PGI</td>
<td>0.65</td>
<td>30.9 μg/L</td>
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<td>PGI/PGII</td>
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<td>1.0</td>
<td>0.94</td>
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<tr>
<td>Hp-ab</td>
<td>0.75</td>
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<td>75%</td>
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AUC: area under the ROC curve.

Conclusions: Apoptotic activity in gastric mucosa increases significantly as a response to H. pylori, and the eradication has a strong potential to suppress the inflammatory process and to normalize apoptosis in pediatric patients.

Abstract no.: W1.5
Accuracy of GastroPanel for Noninvasive Diagnosis of Atrophic Gastritis


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Background: It has been published that GastroPanel might be a helpful tool for the diagnosis of atrophic gastritis measuring four biomarkers in blood: basal gastrin-17 (G-17), pepsinogen I and II (PGI and PGII), and Helicobacter pylori antibodies (Hp-ab).

Aim: To determine GastroPanel’s accuracy for atrophic gastritis diagnosis.

Methods: Prospective, blind, multicenter study including dyspeptic patients. G-17, PGI, and PGII were determined by ELISA (Biohit, Helsinki, Finland). Three antrum and two corpus biopsies were obtained during endoscopy for standard histologic analysis and rapid urease test (RUT). Biopsies were analyzed by a single blind expert pathologist.

Results: Eighty-three patients have been included (76% female, mean age 45 years, 53% H. pylori-positive and 9.6% with atrophic gastritis). Basal concentrations of G-17, PGI, and PGII were determined by ELISA (Biolsit, Helsinki, Finland). Three antrum and two corpus biopsies were obtained during endoscopy for standard histologic analysis and rapid urease test (RUT). Biopsies were analyzed by a single blind expert pathologist.

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AUC: area under the ROC curve.
Conclusion: These results do not show enough evidence in support of the systematic use of GastroPanel for the diagnosis of atrophic gastritis.

Abstract no.: W1.6
H. pylori Antibody Patterns in the German Population Identified with Multiplex Serology

A. Michel, T. Waterboer, M. Kist and M. Pawlita
Department of Genome Modifications and Carcinogenesis, Infection and Cancer Program, German Cancer Research Center (DKFZ), Heidelberg, Germany; National Reference Center for H. pylori, Institute of Medical Microbiology and Hygiene, University Hospital, Freiburg, Germany

Chronic Helicobacter pylori infection is associated with severe gastrointestinal disease including gastric cancer. It induces complex antibody responses that may vary depending on stage and type of infection but currently cannot be characterized adequately. We developed H. pylori multiplex serology based on recombinantly expressed and affinity-purified H. pylori proteins in combination with fluorescent bead technology (Luminex). It allows simultaneous and quantitative detection of antibodies against 15 different H. pylori proteins from strains 26695 and/or G27.

To exemplarily characterize the immune response to H. pylori in a low endemic country, we analyzed 1797 sera representative for the German population (age range 1–82 years). Overall H. pylori seroprevalence within the German population was 48%. Antibodies to omp, vacA, and GroEL were most prevalent. H. pylori seroprevalence increased with age and reached a maximum in males older than 65 years. Among seropositive individuals, immunoreactivity increased with age: the median number of antigens recognized increased from 7.5 (range: 4–13) in the 1- to 14-year olds, to 10.0 (range: 4–15) in the above 65-year olds (p = .002), and antibody titers to GroEL, vacA, hyuA, cag16, catalase, napA, and ureA increased on average 6.1-fold (standard deviation: 4.1). This may reflect a lifelong boosting of the immune response.

With its high-throughput capability (2000 sera can be analyzed per day), H. pylori multiplex serology appears suited for large seroepidemiologic studies assessing prevalence and disease association of H. pylori infection.

Abstract no.: W1.7
Association Between Chronic Atrophic Gastritis and Serum Antibodies to 15 H. pylori Proteins Measured by Multiplex Serology

L. Gao, M. N. Weck, A. Michel, M. Pawlita and H. Brenner
German Cancer Research Center, Heidelberg, Germany

Background: Infection with Helicobacter pylori is a major risk factor for chronic atrophic gastritis (CAG), a precursor lesion of intestinal gastric cancer. Pathogenicity of the bacterium is thought to play an important role in determining extent and severity of the clinical outcome. We aimed to assess the associations between CAG and serostatus of antibodies to 15 H. pylori proteins.

Methods: The analyses were based on 534 cases with serologically defined CAG and 1068 age- and sex-matched controls participating in a population-based study conducted in Saarland/Germany among 9953 men and women aged 50–74 years. A newly developed H. pylori multiplex serology method was used to detect antibodies specific to 15 H. pylori antigens.

Results: Significant associations were observed between seropositivity for all 15 specific antibodies and presence of CAG. Exclusion of severe cases, who might have lost the infection in the course of CAG progression, substantially increased the observed associations. In H. pylori seropositive subjects, cytotoxin-associated gene A (CagA) vacuolating toxin (VacA), helicobacter cysteine-rich protein C (HcpC), and the chaperonin GroEL were identified as independent virulence factors for CAG with adjusted odds ratios (95% confidence interval) of 3.52 (2.01–6.10), 3.19 (1.44–7.05), 4.03 (1.53–10.65), and 2.65 (1.06–6.62) respectively; simultaneous presence of all four independent virulence factors was associated with an 18-fold risk of CAG.

Conclusions: In conclusion, HcpC and GroEL were identified as new independent virulence factors and, in combination with the established virulence factors CagA and VacA, were strongly associated with CAG.

Abstract no.: W1.8
Endoscopic and Histopathologic Characteristics of H. pylori Infection in a Canadian Arctic Hamlet

University of Alberta, Edmonton, Alberta, Canada; Northwest Territories Health and Social Services, Aklavik, Northwest Territories, Canada

The Aklavik H. pylori Project is the start of a community-driven collaboration to investigate Helicobacter pylori infection in northern Canada. Goals are to describe the H. pylori-associated disease burden and reduce related health risks. All residents of the predominantly Aboriginal Hamlet of Aklavik, Northwest Territories (population = 600), were invited to participate. This report describes the endoscopic and histopathologic findings in those who consented to upper gastrointestinal endoscopy in February 2008. Endoscopy equipment was transported temporarily to the local health center. Visiting gastroenterologists performed transnasal ultrathin gastroscopies and obtained gastric biopsies (2 antrum, 2 corpus, 1 incisura). Each biopsy was processed with hematoxylin and eosin and Giemsa stains and evaluated microscopically by a single pathologist using the updated Sydney system. Among 194 participants (aged 10–80 years) who completed endoscopy with biopsies, frequency of endoscopic abnormalities was: 10.4% esophagitis, 2.6% Barrett’s esophagus; 13.8% gastritis; 6.2% gastric erosions; 3.1% gastric ulcers; 6.7% duodenitis; 0.5% duodenal erosions; 0 duodenal ulcers; and 0 gastric cancers. Histopathologic findings appear in the table for Aklavik’s major ethnicities (excluding 8 other, 1 missing).

This project has identified a high-risk population in need of effective strategies for reducing H. pylori-associated health risks.
Workshop 2: Inflammation and Immunity

Abstract no.: W2.1

*H. pylori* Cag Pathogenicity Island-Dependent Downregulation of the Anti-inflammatory Response in Gastric Cells

S. Hofbaur,* T. Wiedemann,* E. Loell,* S. Mueller,† R. Haas* and G. Rieder*

*Max von Pettenkofer Institute, Munich, Germany; †Institute of Pathology, Munich, Germany

**Background:** A persistent gastric colonization of *Helicobacter pylori* is associated with a chronic gastritis characterized by the infiltration of immunoreactive cells. A chronic *H. pylori* inflammation over time is reflected by a dominant Th1-response.

**Aim:** The question arose; how *H. pylori* manipulates the immune system, focusing on the anti-inflammatory response in vivo and in vitro.

**Methods:** Mongolian gerbils were infected with *H. pylori* B128 (WT) or an isogenic cagY-mutant for 2 to 64 weeks. Paraffin sections of antrum and corpus mucosa were graded, and inflammatory markers were measured by quantitative reverse transcription-polymerase chain reaction. In vitro promoter analyses were addressed by applying luciferase-reporter constructs of the chemokines interleukin (IL)-8 and IL-10.

**Results:** WT-infected gastric mucosa revealed a severe inflammation after 8 weeks postinfection; this was not observed with the cagY-mutant strain. The significant up-rise of proinflammatory cytokines (e.g. KC) was associated with a delayed upregulation of the anti-inflammatory cytokine IL-10 in antral mucosa of WT-infected gerbils. The in vitro analysis of these two cytokines revealed a cag pathogenicity island (cag-PAI)-dependent upregulation of the IL-8 promoter as well as a significant downregulation of the IL-10 promoter. The known signaltransduction pathway of IL-8 was confirmed by applying specific inhibitors for NFκB and MEK/ERK pathways; parallel we could show that both pathways are involved in the IL-10 regulation, too.

**Conclusion:** Our data reveal that an intact cag-PAI is not only responsible for an early up-rise of the proinflammatory IL-8 but also for an initial downregulation of the anti-inflammatory IL-10, thus a severe antral inflammation is a direct effect of *H. pylori* virulence factors.

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Abstract no.: W2.2

Macrophage Polarization is Skewed to M1 in Human Atrophic Gastritis

M. Quiding-Järbrink, A. Östberg, S. Raghavan, S. Lundin and M. Sundquist

Department of Microbiology and Immunology, Göteborg, Sweden

*Helicobacter pylori* infection triggers a chronic gastric inflammation that can progress to atrophy and gastric cancer. Classically activated macrophages (M1) expressing iNOS are associated with development of gastric cancer, whereas alternatively activated macrophages (M2) producing CCL18 are associated with prolonged survival in gastric cancer. To investigate macrophage polarization during *H. pylori* infection, mice were orally infected with SS1 and the inflammatory infiltrate of the stomach was analyzed by flow cytometry. The frequency of neutrophils was increased > 10-fold 4 weeks after infection, and the frequency of eosinophils increased 2-fold after 8 weeks. The frequency of gastric macrophages was not changed at these time points. The expression of M2 polarization markers (FIZZ-1, arginase-1, interleukin-10) was not upregulated in the stomach at 4 or 8 weeks of infection. In contrast, the expression of the M1 marker CXCL11, but not iNOS, was upregulated after 8 weeks. Furthermore, mice that were immunized intranasally with cholera toxin and *H. pylori* lysate upregulated the expression of both iNOS and FIZZ-1 after challenge. Similarly, subjects with asymptomatic *H. pylori* infection showed an elevated expression of human M2 markers (CCL17, CCL18, the mannose receptor) and M1 markers (iNOS, CXCL11) in the antrum. Individuals with atrophy expressed iNOS (15-fold) compared to asymptomatic carriers, whereas markers of M2 polarization were similarly expressed. These findings show that polarization of gastric macrophages is skewed to M1 in human atrophic gastritis, and that protective immunization also affects macrophage polarization.

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<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th><em>H. pylori</em>+</th>
<th>Severe inflammation</th>
<th>Atrophy</th>
<th>Intestinal metaplasia</th>
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<td>70.2%</td>
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<td>22.2%</td>
<td>11.1%</td>
<td>11.1%</td>
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<tr>
<td>All <em>H. pylori</em>+</td>
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<td>–</td>
<td>43.4%</td>
<td>20.9%</td>
<td>10.9%</td>
</tr>
<tr>
<td>All participants</td>
<td>194</td>
<td>66.5%</td>
<td>28.9%</td>
<td>13.9%</td>
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</tbody>
</table>

Inuit 114 70.2% 31.6% 14.0% 9.6%
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Non-aboriginal 18 22.2% 11.1% 11.1% 5.6%
All *H. pylori*+ 129 – 43.4% 20.9% 10.9%
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Workshop 2: Inflammation and Immunity

Abstract no.: W2.1

**H. pylori Cag Pathogenicity Island-Dependent Downregulation of the Anti-inflammatory Response in Gastric Cells**

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M. Quiding-Järbrink, A. Östberg, S. Raghavan, S. Lundin and M. Sundquist
Department of Microbiology and Immunology, Göteborg, Sweden

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Abstract no.: W2.3
Critical Role of Toll-like Receptor in the H. pylori-Induced Immune Response

C. Prinz, L. Rad and R. Rad
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Background and Aims: Toll-like receptors (TLR) are responsible for the recognition of infection, leading to induction of adaptive immunity through activation of antigen-presenting cells (APCs). Methods: We analyzed molecular mechanisms of the Helicobacter pylori-induced APC activation in vitro and investigated the influence of Myd88 signaling on the phenotype of adaptive immunity to H. pylori in a murine infection model.

Results: The adaptor protein Myd88 mediates TLR, interleukin (IL)-1, and IL-18 signaling. Dendritic cells (DCs) from wild-type, IL-1R(–/–), and IL-18R(–/–) mice responded to H. pylori with secretion of proinflammatory cytokines and upregulation of major histocompatibility complex II and costimulatory molecules. In Myd88(–/–) DCs these processes were impaired profoundly, showing that TLR-dependent H. pylori-sensing affects DC activation. Analysis of the H. pylori-specific DC transcriptome revealed that large parts of the bacteria-induced transcriptional changes depended on Myd88 signaling, comprising numerous genes involved in crucial steps of immune regulation, such as DC maturation/differentiation, antigen uptake/presentation, and effecter cell recruitment/activation. The impaired ability of Myd88(–/–) DCs, B cells, and macrophages to mount a proinflammatory response to H. pylori in vitro was reflected in vivo by reduced gastric inflammation and increased bacterial colonization in Myd88-deficient mice. Furthermore, Helicobacter-specific IgG2c/IgG1 ratios were reduced in Myd88(–/–) animals, suggesting the involvement of the Myd88-dependent pathway in the instruction of adaptive immunity toward a T helper cell type 1 phenotype.

Conclusions: A principal pathway by which DCs sense H. pylori and become activated is the TLR-dependent signaling cascade. In vivo, Myd88 signaling affects adaptive immunity to the bacterium.

Abstract no.: W2.4
Adaptive Cellular Response to H. pylori-induced Prolinflammatory Injury

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We hypothesized adaptive response after Helicobacter pylori can explain why a few develop clinical diseases while most remain asymptomatic. First, we documented the serial changes (0.5, 1, 2, 4, 8, 12, 24 hours) of COX-2, interleukin-8, iNOS, HSPs, and HO-1 in AGS cells infected with H. pylori. Significantly increased inductions of damaging genes with activation of p-ERK1/2, c-jun, NF-κB, and AP-1 and simultaneous incremental inductions of defensive genes with activation of nrf-2 and ARE were noted. There was significant correlation between cytotoxicity with H. pylori infection and cancellation of GSH and HSP. We moved to animal experiments to validate in vitro results. For proving the ARE activation after H. pylori, we infected H. pylori to ARE-hPAP+/- and ARE-hPAP-/- transgenic mice for 16 weeks and found H. pylori-induced inflammation elevates ARE activation in mouse stomach. To confirm nrf-2 activation after H. pylori, we infected H. pylori to nrf-2-/- and nrf-2+/- mice for 20 weeks and found that H. pylori-induced inflammation was aggravated in nrf-2-/- mice compared with nrf-2+/- and nrf-2+/- mice. As for clinical relevance, we compared the expressions of COX-2, HO-1, and nrf-2 in samples from chronic gastritis patients according to H. pylori status. Significantly higher expressions of COX-2, HO-1, and nrf-2 were noted in patients with H. pylori (+) chronic gastritis than H. pylori (-). In serial proteomic analysis, we identified proteomes involved in adaptive response that could be the biomarkers predicting the progress of H. pylori-chronic gastritis. Adaptive cellular response to H. pylori played contributions to either host defense or the biomarker.

Abstract no.: W2.5
Genotoxicity Associated to the H. pylori Strains B38, a MALT Lymphoma Derivative, in the Mouse Model

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Helicobacter pylori is until now the only oncogenic bacterium. During its persistence in the host, it promotes genetic instabilities, considered as the first events at the origin of malignancies as gastric cancer lesions and lymphomagenesis. The goals of the present study are to identify the mutagenic response associated with the H. pylori MALT lymphoma strain derivative B38; and to investigate the host systems involved in this genotoxic response, focusing on the activation-induced cytidine deaminase (AID), mainly responsible for chromosomal translocations in immunoglobulin (Ig) genes. AID is a nucleotide editing enzyme also able to induce mutations in non-Ig genes by cytosine deamination, leading to GC->AT transitions if not repaired.

The H. pylori strain B38 was adapted to the mouse stomach and used in the “Big Blue” mutagenesis assay. Genetic mutation frequency and the associated mutation spectra were determined, and the AID expression was investigated. After 6 months of infection, a 5-fold increase of the gastric mutation frequency was measured in H. pylori B38 infected-mice as compared to the control, similarly to what we previously observed with the H. pylori strain SS1. The B38-induced mutation spectra are mainly composed of GC->AT transitions. An induction of the AID expression was evidenced in the gastric mucosa of H. pylori B38 infected-mice, by Western blot analysis.

The induction of genetic instabilities and mutations appeared as a common properties of the H. pylori strains. Experiments are in progress to investigate the involvement of AID in the genotoxic host response induced by the H. pylori infection.
Abstract no.: W2.6
Vaccination Against *H. pylori* with Gamma-Glutamyltranspeptidase

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*Helicobacter pylori* is the most widespread bacterial infection, affecting half of the world's population and causing peptic ulcers and gastric cancer. Although big efforts have been initiated to develop a vaccine against this pathogen none of these experimental approaches led to successful results in human studies and thus failed to generate an approved vaccine in humans. It is unclear if this is due to the antigens used, the vaccine formulations tested to induce protective immunity or the laboratory infection models applied. Our group described a virulence factor of *H. pylori*, the *H. pylori* gamma-glutamyltranspeptidase (HPgGT) that inhibits the proliferation of T-cells and thus prevents the generation of an effective immune response. We used HPgGT in an experimental mouse infection model for a novel vaccination approach. Immunization with this antigen induces strong antibody responses, which blocks its enzymatic activity, thereby counteracting the immunosuppressive activity of HPgGT. Different formulations and routes of application were tested, revealing a need for mucosal immunization. As HPgGT is a secreted protein, HPgGT-specific immune responses against a high variety of strains are being identified by mass spectrometry. High immunogenic protein expressed in at least 65% of the strains among strains included in the study. From these, we selected 11 highly immunogenic protein expressed in at least 65% of the strains which are being identified by mass spectrometry.

So far, all the developed anti-*H. pylori* vaccines, most of which uses antigens proteins known to be involved in the pathogenesis, have failed. Therefore, the identification of the proteins revealed in this study will disclose new candidates for the construction of an efficient DNA-based broad-spectrum vaccine. Moreover, identification of strain-specific immunogenic protein spots will bring more answers to the pathogenesis of this organism.

We thank Professor Mónica Oleastro (INSA, Portugal) for the strains. Work supported by PTDC/BIO/69242/2006 research grant. IV is recipient of SFRH/BD/38634/2007 doctoral fellowship.

Abstract no.: W2.7
Application of Immunoproteomics Technology in the Design of a Multivalent Vaccine Against *H. pylori*

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*Helicobacter pylori* infection is the second most common infection among world population. Due to high genetic variability between strains, the development of an effective vaccine against *H. pylori* requires the identification of antigens commonly expressed among them.

Protein extracts from a total of 11 different strains, isolated from patients with normal mucosa, epigastric pain, gastritis, peptic ulcer, and gastric cancer, were analyzed by two-dimensional gel electrophoresis (2DE). Proteins were then electrophoretically transferred onto nitrocellulose membranes, and those that are immunogenic were revealed by incubation with a pool of antibodies against *H. pylori* (Biodesign). Immunodetection was performed with enhanced chemiluminescence (ECL kit, Pierce). 2DE-blots digitalized images were analyzed using ImageMaster™ 2D-Platinum software (Geneva-Bioinformatics, SA) in order to select the immunogenic protein spots that are commonly expressed among strains included in the study. From these, we selected 11 highly immunogenic protein expressed in at least 65% of the strains which are being identified by mass spectrometry.

Compared to conventional vaccines, multigenic DNA vaccines present theoretical advantages for the large-scale medical eradication of *Helicobacter pylori* as they may elicit both host's humoral and cellular responses against a high variety of strains.

Based on their pathogenic relevance, three *H. pylori* antigens were chosen for the DNA-vaccine construction: the chaperonin GroEL, which being a member of the heat-shock proteins' family, is highly expressed in response to stress such as inflammation; the external membrane protein HomB which is a relevant virulence factor involved with inflammatory response and adherence, and the highly virulent marker VacA protein, as several studies indicate that this protein strongly contributes to the *H. pylori* persistence by suppressing the host adaptive immunity. Instead of using their whole sequence, the plasmid backbone of our DNA-vaccine construction contains the nucleotide sequence coding for 50 amino-acid residues long fragments, each being representative of the most conserved and immunogenic region of each of the three target proteins. Moreover, in order to minimize the interference between adjacent antigens and to ensure fusion-protein stability, a peptide linker was included between them. In vitro transfection efficacy is being evaluated using the AGS cell line.

The construction of the multigenic DNA vaccine that supports balanced Th1/Th2 responses against the relevant virulence factors GroEL and HomB, and that simultaneously counteracts one of the most relevant evasion mechanisms of this bacterium to the host immune system mediated by VacA, will be discussed.

Work supported by PTDC/BIO/69242/2006 (FCT) research grant. Carvalho is recipient of SFRH/BD/23902/2005 (FCT) doctoral fellowship.
Workshop 3: Gastric Cancer I

Abstract no.: W3.1
H. pylori Infection and Gastric Cancer Risk: Evaluation of 15 H. pylori Proteins Determined by Novel Multiplex Serology

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Infection with Helicobacter pylori is a major cause of gastric cancer (GC). The association likely has been underestimated in the past due to disease-related clearance of the infection. On the other hand, only a minority of the infected individuals develop GC, and better risk stratification is therefore highly desirable. We aimed to assess the association of GC with antibodies to 15 individual H. pylori proteins, determined by novel multiplex serology, to identify potentially relevant risk markers. The analyses are based on 123 GC cases aged 50–74 years and 492 age- and sex-matched controls from Saarland, Germany. Eight of the antibodies were significantly associated with noncardia GC and seven of them were significantly related to GC at any site. More pronounced associations were observed for noncardia GC, adjusted odds ratios (95% confidence intervals) ranged from 1.60 (1.01–2.54) for HyuA to 5.63 (3.20–9.91) for CagA. A dose–response relationship was found between the number of seropositivities and GC (p < .001). Seropositivities of CagA and GroEL were found to be independent predictors of GC which were strongly related to GC risk in a dose–response manner (p < .001). In conclusion, GroEL was identified as a new independent risk marker that may contribute to enhanced quantification of H. pylori-related GC risk.

Abstract no.: W3.2
Dual Regulation by AP Endonuclease-1 Inhibits Gastric Epithelial Cell Apoptosis During H. pylori Infection

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Background: Human AP endonuclease-1 (APE-1), a key enzyme involved in repair of oxidative DNA base damage, is an important transcriptional coregulator. We previously reported that Helicobacter pylori infection induces apoptosis and increases APE-1 expression in human gastric epithelial cells (GEC). Although both the DNA repair activity and the acetylation-mediated transcriptional regulation of APE-1 are required to prevent cell death, the exact mechanisms of infection-mediated inhibition of apoptosis by APE-1 are unclear.

Methods: Stable shRNA APE-1 suppressed as well as empty vector and nontransfected AGS GEC were used to assess extrinsic and intrinsic pathways of apoptosis. Mutations of APE-1 deficient in DNA repair activity or acetylation function were employed to determine the specific functions of APE-1 involved in regulating H. pylori-mediated apoptosis.

Results: Increased caspase 3 and cleaved PARP were found in APE-1 suppressed cells during H. pylori infection. Both caspase 9-mediated mitochondrial and caspase 8-mediated extrinsic pathways of apoptosis were shown to be involved. Overexpression of wild-type APE-1 in these cells reduced infection-induced apoptosis; however, mutation of the DNA repair or acetylation functions of APE-1 did not have this inhibitory effect, suggesting that both APE-1 functions modulate programmed cell death.

Conclusions: We show for the first time that the DNA repair activity inhibits the mitochondrial pathway of apoptosis while its acetylation function inhibits the extrinsic pathway during H. pylori infection. Thus, our findings establish a dual regulatory function of APE-1 in H. pylori-mediated GEC apoptosis. Our results have implications for understanding mechanisms of H. pylori-associated gastric carcinogenesis.

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Abstract no.: W3.3
Are First Degree Relatives of Gastric Cancer Patients at an Increased Risk for Gastric Cancer? A Meta-analysis

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Background: Helicobacter pylori is believed to predispose to gastric cancer by inducing precancerous changes, i.e., atrophy and intestinal metaplasia (IM). First-degree relatives of patients with gastric cancer might be at an increased risk of developing gastric cancer. However, this evidence is based on scattered individual studies.

Aims: The aim of this study was to examine the risk of first-degree relatives developing gastric cancer by meta-analyzing all relevant studies.

Methods: Extensive English language medical literature searches for human studies were performed up to the end of April 2009. Inclusion and exclusion criteria were identified, and in eligible studies data on three parameters, i.e., H. pylori prevalence, atrophy, and IM, were extracted. Pooled estimates [odds ratio (OR) with 95% confidence intervals (CI)] were obtained using either the fixed or random-effects model as appropriate.

Results: Of 149 initially identified studies, seven studies, from various countries, fulfilling the inclusion criteria, examined the risk of first-degree relatives developing gastric cancer (n = 1095) in comparison to controls (n = 1248). For H. pylori prevalence, the pooled OR with 95% CI was 1.90 (1.30–2.79) and the test for overall effect Z was 3.34 (p = .001). The respective values for atrophy and IM were 4.14 (2.55–6.71), Z = 5.67 (p = .00), and 2.67 (1.87–3.81), Z = 5.41 (p = .0001) respectively.

Conclusion: First-degree relatives of patients with gastric cancer are at an increased risk of developing gastric cancer. Consequently upper gastrointestinal endoscopy with H. pylori detection and prophylactic eradication of the infection should be offered to such individuals.
Abstract no.: W3.4

H. pylori Infection Induces Bone Marrow Stem Cell Recruitment and Homing in the Gastric Epithelial Mucosa in a Mouse Model

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Background: It has been established that Helicobacter pylori infection is responsible for more than two-thirds of gastric cancers. Recent studies on mice infected with Helicobacter felis have established a new model of gastric carcinogenesis, involving bone marrow mesenchymal stem cells (MSC) at the origin of gastric preneoplastic lesions and carcinoma (Houghton et al., Science 2004).

Aim: The aim of this study was to determine if MSC are also at the origin of gastric carcinogenesis in response to an infection with H. pylori.

Methods: To achieve this, we developed a model of gastric carcinogenesis in mice infected with H. pylori, allowing the specific detection of bone marrow stem cells. Chimera mice, generated using wild-type mice lethally irradiated and reconstituted with bone marrow from transgenic mice expressing the green fluorescent protein (GFP), were infected with three strains of H. pylori supposedly able to colonize mice, and two “mouse adapted” strains. The chronic inflammation and the pathologic evolution of the gastric mucosa as well as MSC recruitment were followed from 15 weeks to 75 weeks postinfection.

Results: Preliminary results (two of five strains) indicate that H. pylori infection induces chronic inflammation and mucosal metaplasia as early as 15 weeks postinfection, and pseudointestinal metaplasia and dysplasia after 1 year. After 1 year, GFP-positive epithelial cells forming epithelial glands of the gastric mucosa were found in mice infected with H. pylori but not in noninfected control mice. Further analyses after 75 weeks postinfection will determine whether recruited MSC are involved in high-grade dysplasia and carcinoma.

Conclusions: Our results suggest that H. pylori impairs central DNA repair mechanisms, inducing a transient mutator phenotype, rendering gastric epithelial cells vulnerable to the accumulation of genetic instability and thus contributing to gastric carcinogenesis in infected individuals.

Abstract no.: W3.5

H. pylori Infection Induces Genetic Instability of Nuclear and Mitochondrial DNA in Gastric Cells

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Aim: Helicobacter pylori is a major cause of gastric carcinoma. To investigate a possible link between bacterial infection and genetic instability of the host genome, we examined the effect of H. pylori infection on mismatch repair pathway (MMR). Moreover, various types of genetic instabilities in the nuclear and mitochondrial DNA (mtDNA) were examined.

Experimental Design: We observed the effects of H. pylori infection on a gastric cell line (AGS) and on C57BL/6 mice. In AGS cells, the effect of H. pylori infection on MMR was analyzed by an activity assay. Microsatellite instability (MSI) was also analyzed in AGS cells using the HNPCC MSI Test Kit. In mice, CA repeat instabilities were examined by Mutation Detection Enhancement gel electrophoresis. Mutation spectra in AGS cells were determined by polymerase chain reaction (PCR) sequencing, and mtDNA depletion was evaluated by real-time PCR.

Results: Following H. pylori infection, MMR activity is downregulated. Moreover, H. pylori induces genomic instability in nuclear CA repeats in mice, but is not able to lead to MSI in vitro, at least with the experimental setting used. In AGS cells, H. pylori is able to induce mtDNA mutations in coding genes and mtDNA depletion.

Conclusions: Our results suggest that H. pylori impairs central DNA repair mechanisms, inducing a transient mutator phenotype, rendering gastric epithelial cells vulnerable to the accumulation of genetic instability and thus contributing to gastric carcinogenesis in infected individuals.

Abstract no.: W3.6

Role of DNA Hypermethylation in the Downregulation of USF1/2 Transcription Factors Expression and their Target Gene the TERT in H. pylori Infection

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Helicobacter pylori is responsible for gastric cancer and MALT lymphoma. Upstream stimulatory factors (USF1, USF2) are transcriptional regulators downregulated in some cancers. We previously described a decrease of USF2 in mice chronically infected with H. pylori. Here, we studied, during H. pylori infection, the regulation of USF1, USF2, and their target gene TERT coding for the main component of telomerase that maintains chromosome stability.

Gastric epithelial cells infected with H. pylori strain B38 (from a patient with MALT lymphoma) showed a decrease in USF1/2 RNA and protein levels and in TERT transcription, thereby decreasing telomerase activity. USF1/2 downregulation is associated with a decrease of the complex binding to the TERT promoter. However, USF1/2 overexpression did not restore TERT gene expression during H. pylori infection, suggesting another mechanism. As epigenetic mechanisms such as promoter DNA hypermethylation lead to gene silencing, we used a DNA methylation inhibitor 5′-azacytidine and showed a restoration of USF1/2 RNA expression and TERT. We explored the methylation status at the promoter level of USF1/2 and TERT in gastric tissues from 18-month
infected and non-infected mice and demonstrated a higher methylation at the CpG islands and in the vicinity of E boxes. In conclusion, H. pylori infection induces a DNA hypermethylation, involved in the downregulation of USF1/2 and their target gene TERT. Given the role of USF1 and USF2 as tumor-suppressor genes and telomerase in the maintenance of chromosome integrity, the mechanisms described here may play an important role in driving the infection towards the development of malignancies.

Abstract no.: W3.7
H. pylori Induces Genomic DNA Methylation in Human Gastric Cell Lines

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Restriction and modification systems (RMS) are prokaryotic enzymatic systems of variable complexity. RMS are comprised of at least two proteins: a phosphodiesterase that hydrolyzes two phosphodiester bonds in a specific dsDNA sequence and a methyltransferase that transfers a methyl group from S-adenosylmethionine to adenine or cytosine in the cognate phosphodiesterase-specific DNA sequence.

In mammals, the methylation of cytosine residues is the predominant post-replication base modification. Defects in methylation of total DNA or particular DNA sequences have been shown to be associated with carcinogenesis, possibly as a facilitating factor for aberrant under- or overexpression of genes linked to cancer.

RMS are comprised of at least two proteins: a phosphodiesterase that hydrolyzes two phosphodiester bonds in a specific dsDNA sequence and a methyltransferase that transfers a methyl group from S-adenosylmethionine to adenine or cytosine in the cognate phosphodiesterase-specific DNA sequence.

There are six Helicobacter pylori genomes completely sequenced, all with a large number of methyltransferases: the lowest (28) in strain 26695 and the highest (34) in strain G27. Most of these methyltransferases are active because they are part of an RMS and the phenotype mod-ires+ is lethal. Several are cytosine methyltransferases and there is evidence that most of H. pylori strains are methylated in the “GCRC” sequence (HhaI recognition sequence).

We did cocultures of two H. pylori strains with two human gastric cell lines (AGS and N87). After isolation of genomic DNA from the cell lines, we tested for alterations on the methylation of some gene promoters, using methylation-sensitive polymerase chain reaction and general genomic methylation using several restriction endonucleases. The results confirm that the presence of H. pylori increases the genomic methylation of both human cell lines.

The impact of RMS on eukaryotic DNA methylation should be further investigated.

Abstract no.: W3.8
A Combination of Sulindac and Antimicrobial Eradication of H. pylori Prevents Progression of Gastric Cancer in Hypergastrinemic INS-GAS Mice

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Helicobacter pylori infection causes severe dysplasia manifested as gastrointestinal intraepithelial neoplasia (GIN) after 28 weeks post-H. pylori infection (WPI) in cancer-prone, hypergastrinemic male INS-GAS mice. We examined the efficacy of the nonsteroidal anti-inflammatory drug sulindac (400 ppm in drinking water) alone, the CCK2/gastrin receptor antagonist YM022 (45 mg/kg/week) alone, and sulindac or YM022 combined with H. pylori eradication therapy to prevent H. pylori-associated gastric cancer in male INS-GAS mice. Treatments started at 22 WPI, and mice were euthanized at 28 WPI. In uninfected mice, all treatments significantly delayed development of spontaneous GIN (p < .05). In H. pylori-infected mice, sulindac alone or YM022 alone had no protective effect on H. pylori-associated GIN. Importantly, sulindac exacerbated the severity of H. pylori-associated gastritis despite decreased gastric PEG levels. However, sulindac combined with H. pylori antimicrobial eradication reduced the incidence of GIN (p < .05), whereas YM022 combined with antimicrobial eradication did not reduce GIN. In infected mice, sulindac or YM022 treatment did not alter gastric expression of the proinflammatory cytokines Ifn-γ and Tnf-α and the growth factor, Reg1. Sulindac or YM022 combined with antimicrobial eradication downregulated mRNA levels of Ifnγ, Tnfa, and Reg1 (p < .05). We conclude that sulindac enhances H. pylori gastritis and may promote inflammation-mediated gastric carcinogenesis. The combination of sulindac and antimicrobial H. pylori eradication was beneficial for reducing proinflammatory cytokine mRNA in the stomach and preventing progression from severe dysplasia to gastric cancer in H. pylori-infected INS-GAS mice.
Study investigated the effects of encounter bile which has potent antibacterial activity. This human samples has been correlated with cholangiocarcinoma. *Massachusetts Institute of Technology, Cambridge, Massachusetts, USA; †Hospital Ramon y Cajal, Madrid, Spain

We recently described helicobacter-associated progressive, proliferative, and dysplastic typhlocolitis in aging (18–24 months old) Syrian hamsters. Other pathogens associated with typhlocolitis in hamsters, Clostridium difficile, Lawsonia intracellularis, and Giardia spp. were not indentified. The presence of Helicobacter-genus-specific DNA was noted by polymerase chain reaction (PCR) in cecal and paraffin-embedded liver samples from aged hamsters, using Helicobacter PCR-specific primers. By 16SrRNA analysis, the Helicobacter spp. isolated from the liver tissue was identical to the cecal isolates of hamsters. The six hamster 16SrRNA sequences form a genotypic cluster, most closely related to Helicobacter sp. Flexispira taxon 8, part of the *H. bilis/H. cinaedi* group. Livers from aged helicobacter-infected hamsters showed varying stages of predominantly porto-centric and to a lesser extent, perivenular fibrosis. Within nodules, there was cellular atypia consistent with nodular dysplasia. The livers also exhibited a range of chronic active portal/interface, and lobular inflammation with significant portal hepatitis was present. The inflammation was composed of a mixture of lymphocytes, neutrophils, and macrophages indicative of its chronic-active nature in these aged hamsters infected with Helicobacter spp. The isolation of novel Helicobacter sp. and their identification by PCR from the diseased livers of aged hamsters and their taxonomic classification as belonging to Helicobacter sp. taxa *bilis* strengthen the argument that *H. bilis*, and closely related *Helicobacter* spp., play an etiologic role in hepatobiliary disease in both animals and humans.

**Abstract no.: W4.2**

*Changes in Protein Expression of Human Hepatoma Cells Induced by Helicobacter bilis are Modulated by Bile*

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*Helicobacter bilis* colonizes the gall bladder and liver of mice causing chronic inflammation. The presence of *Helicobacter* species DNA in human samples has been correlated with cholangiocarcinoma and hepatocarcinoma. In their various niches, these bacteria encounter bile which has potent antibacterial activity. This study investigated the effects of *H. bilis* on the morphology, proliferation, and protein expression of human Huh7 cells in the presence of human bile.

Semiconfluent Huh7 cell cultures were incubated in the presence of 0–1% bile concentrations for 48 hours under a microaerobic atmosphere or under the same conditions in cocultures with various inoculum densities of *H. bilis* with or without bile. Relative to control cultures without bile, colony-forming units per milliliter (cfu/mL) of cultures declined by 1.4-log and 2.6-log at 0.05% and 0.1% bile concentrations, respectively; there was no observable effect of bile on the morphology and proliferation of the hepatoma cells. In Huh7/*H. bilis* cocultures, hummingbird morphology and gradual reduction in the number of live Huh7 cells at increasing bacterial densities from $10^2$ to $10^6$ cfu/mL were observed. Protein expression of Huh7 cells was investigated employing 2D-PAGE and identified by liquid chromatography-mass spectrometry.

*H. bilis* induced in Huh7 cells showed different responses depending on whether or not human bile was present in cultures. For example, peroxiredoxins 2 and 3 were downregulated in the presence of bile with or without *H. bilis*, respectively; whereas in cocultures without bile, peroxiredoxin 3 was upregulated by Huh7 cells.

Overall, results showed that bile could modulate the response of liver cells to the presence of *Helicobacter* spp.

**Abstract no.: W4.3**

*Members of the Helicobacteraceae Reside in Intestinal Tract of Children and may be Associated with Crohn’s Disease*

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**Introduction:** Although evidence exists to support the role of bacteria in Crohn’s disease (CD) to date no definitive association has been established between any bacterium or bacterial group and CD. Given that enterohepatic *Helicobacter* species reside in the mucus layer of the intestinal tract we hypothesized that these bacteria may play a role in the initiation of CD.

**Methods:** Intestinal biopsy specimens from 67 children with newly diagnosed CD and 93 non-inflammatory bowel disease controls were collected. To investigate whether members of the Helicobacteraceae were present in these biopsy samples, Helicobacteraceae-specific polymerase chain reaction (PCR) was conducted. All PCR-positive samples were sequenced to putative identity the bacterium, and in a subset fluorescent in situ hybridization (FISH) was conducted to determine their location. All biopsy specimens were cultured in an attempt to isolate members of the Helicobacteraceae.

**Results:** Helicobacteraceae DNA was detected in 49% of CD children, which was significantly higher than that in controls [24.7%; (p < .01)]. Sequencing of PCR products showed these to be similar to *Wolinella succinogenes*, *H. trogontum*, *H. ganmani*, *H. bilis*, and *H. cinaedi*.
**Abstract no.: W4.4**

Liver Tumor Promotion in Constitutive Androstane Receptor Knockout Mice Chronically Infected with *Helicobacter hepaticus*

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The nuclear receptor constitutive androstane receptor (CAR) regulates the expression of enzymes involved in endobiotic/xenobiotic metabolism. CAR is essential for liver tumor promotion by phenobarbital in mice. The mechanism by which *Helicobacter hepaticus* promotes liver tumors in mice is not known. We investigated the role of CAR in liver tumor promotion by *H. hepaticus*. Twenty two CAR−/− (KO) and 23 CAR+/+ (wild-type, WT) male mice (C3H/HeN) received a single intraperitoneal injection of the genotoxic carcinogen diethylnitrosamine (DEN) at 5 weeks of age. Another group consisting of 21 KO and 20 WT male mice did not receive DEN. The two groups (with and without DEN) were subsequently stratified into four subgroups (two KO and two WT) and orally inoculated with either *H. hepaticus* or sterile media at 8 weeks of age. Mice were euthanized at 50 weeks postinoculation, complete necropsies were performed, and samples were collected for histologic evaluation. Chronic infection with *H. hepaticus* induced hepatitis in WT and KO mice with or without DEN. However, relative to *H. hepaticus*-infected WT mice, *H. hepaticus*-infected KO mice exhibited the following statistically significant differences: increased number of liver lobes with dysplasia/neoplasia with (*p* < .0001) or without DEN (*p* < .002), increased number of dysplastic and neoplastic liver lobes with inflammation (*p* < .02) and increased multiplicity of preneoplastic liver lesions (*p* < .02) (without DEN), and increased multiplicity of liver tumors (*p* < .004) with DEN. Our findings indicate that CAR protects the liver from tumor promotion by *H. hepaticus*, suggesting a mechanism involving endobiotic/xenobiotic metabolism.

**Abstract no.: W4.5**

Gene Expression Profile of Huh7 Human Hepatocarcinoma Cells Infected with *H. pullorum*

Q. V. Tu, R. Williams, H. Mitchell and G. L. Mendz

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Increasing evidence suggests that in instances of hepatocellular carcinoma (HCC) of unknown etiology, bacterial infection of the genus *Helicobacter* could be a significant risk factor. To investigate this hypothesis the effects of *Helicobacter pullorum* on the transcriptome of the Huh7 human cancer cell line were characterized by coculturing cells with bacteria (tests) and identifying the changes in the transcriptome relative to that of cells cultured without bacteria (controls). Huh7 cells were incubated for 30 minutes, 8 hours, 24 hours, and 48 hours, and at each time point, cells from the control and test samples were harvested. Microarray experiments were performed in duplicate using Affymetrix® Human Gene 1.0 ST Arrays.

A total of 558 genes were found, and the pathways and networks to which they belong were determined using Ingenuity Pathway Analysis. A total of 14 canonical pathways (*p* < .005) and nine regulatory networks (score ≥ 28) were modulated by *H. pullorum* during the 48 hours period. The expression of 24 cancer-related genes was observed to be among the most upregulated by the presence of *H. pullorum*. About 40% of these are related to HCC, including genes involved in cell proliferation (WDR16, WNT5A), regeneration of hepatocytes (IKBBK), invasion of carcinoma cells (ITGB4, PLAUR, AMACR), metastasis (TLR6, LASP1), and cell viability or apoptosis (FASLG). The data provided evidence that the presence of the bacterium modulates the expression of genes involved in carcinogenesis, and identified potential molecular targets for future studies aimed at understanding the relationship between HCC and *Helicobacter* infection.

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**Abstract no.: W4.6**

Serologic Analysis of *Helicobacter hepaticus* Infection in Children with Hepatobiliary Diseases


Department of General Medicine and Community Health Science, Hyogo College of Medicine, Nishinomiya, Japan; Department of Pediatrics, Japan Labour Health and Welfare Organization Wakayama Rosai Hospital, Wakayama, Japan; Gastrointestinal Research Sagami Research Laboratories, Wakamoto Pharmaceutical Co., Ltd., Kanagawa, Japan; Department of Microbiology, Yamaguchi University School of Medicine, Ube, Japan; Department of Gastroenterology, Hirosaki University School of Medicine, Hirosaki, Japan

**Aim:** Several studies have also suggested the possibility that infection of *Helicobacter hepaticus* plays a role in the pathogenesis...
H. pylori Eradication Leads to an Increase in Platelet Count and a Reversal of Proinflammatory Cytokine Profile in a Subset of Patients with Chronic Immune Thrombocytopenic Purpura


Although there are evidences that T cells contribute to the pathogenesis of immune thrombocytopenic purpura (ITP) and in a subset of Helicobacter pylori-infected ITP patients a recovery of the platelet count occurs after the bacterium eradication, the mechanisms involved in the ITP pathogenesis associated with H. pylori infection are still unknown. Thus, we evaluated the serum cytokine levels in 124 adult ITP patients and 100 H. pylori-negative patients. Otherwise, the levels of proinflammatory cytokines IL-1α, IL-1β, IL-6, IL-8, IL-12p70, IFN-γ, and TNF-α (p < 10⁻⁴) decreased and the modulatory cytokines (IL-10, p < 10⁻³; TGF-β, p = .05) increased in the patients who had improvement of the platelet count, in contrast to the nonresponders. High concentration of TGF-β before treatment was the only predictive factor for platelet number recovery (logistic analysis). Concluding, we demonstrated that a proinflammatory profile was strongly associated with ITP. This profile dramatically changed after H. pylori eradication in the group of responder patients (CNPq/FAPEMIG).

Abstract no.: W4.7

H. pylori Eradication Therapy for H. pylori Infection and Mortality from Cardiovascular and Other Diseases

T. U. Kosunen, E. Pukkala, S. Sarna and H. Rautelin

Abstract no.: W4.8

Eradication Therapy for H. pylori Infection and Mortality from Cardiovascular and Other Diseases

T. U. Kosunen, E. Pukkala, S. Sarna and H. Rautelin

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Abstract no.: W5.1
Identification of a Lactoferrin-Binding Protein in H. pylori that is Required for Colonization

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Introduction: Helicobacter pylori thrives in the iron-restricted gastric environment but must acquire host iron to do so. Host lactoferrin (Lf) sequesters free iron in the gastric mucosa as an innate defence mechanism against infection, yet Lf serves as an iron source for H. pylori. This study was undertaken to identify the Lf-binding protein (Lf-BP) in H. pylori and to evaluate its role in colonization.

Methods: H. pylori was cultured under iron-restricted conditions and incubated with human Lf coupled to a crosslinker to biotin-tag the putative H. pylori Lf receptor to facilitate enrichment and identification. A mutant in H. pylori SS1 deficient in expression of the Lf-BP was generated. Gerbils were inoculated with gavage with SS1, the mutant or broth (control). Eight weeks postinoculation the gastric mucosa was recovered for quantitative culture.

Results: A biotinylated Lf-BP was detected by Western blot analysis of H. pylori proteins coincubated with the Lf-crosslinker complex. Mass spectrometric analysis revealed that a ferric-iron transporter was the Lf-binding component in H. pylori and that inactivation of the corresponding gene resulted in abrogation of Lf-binding activity. The mutant was unable to grow in Lf-supplemented medium, whereas the parental strain did. Four of five SS1 inoculated gerbils were culture positive. Five gerbils inoculated with the mutant were all culture negative.

Conclusion: H. pylori can efficiently acquire ferric iron from the host via the Lf-BP and this molecule appears to be essential for colonization of gerbils, suggesting a role for this protein in H. pylori iron homeostasis.

Abstract no.: W5.2
A New Family of D,D-carboxypeptidases Involved in Bacterial Pole Formation in H. pylori

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Peptidoglycan is the major component of the bacterial cell wall and it plays a crucial role in shape determination. During growth and cell division, the bacteria require enzymes, like hydrolases, to remodel this peptidoglycan. Bioinformatic analysis of the genome of Helicobacter pylori identified only three putative hydrolases, since partially characterized. This study shows that Hp506, an uncharacterized protein belonging to the β-lytic metallopeptidase family, is a new hydrolase involved in peptidoglycan remodeling.

First we observed the effect of the deletion of the Hp506 gene by scanning electron microscopy. The gene deletion led to multiple but functional poles, suggesting that Hp506 was involved in elongation and new bacterial pole determination. Moreover, the overexpression of the protein resulted in the transformation of bacilli into cocci, confirming the requirement of Hp506 for the bacterial shape. In addition, the high-performance liquid chromatography analysis of the muropeptide composition of the mutant, showed that Hp506 is a D,D-carboxypeptidase, contrary to the other peptidases from the same family. Indeed, it is able to cut between the latests amino acids of the peptide chain, like some low-molecular-weight PBPs, whereas H. pylori does not own such proteins. Finally, we interestingly reported that Hp506 modulated the susceptibility of H. pylori to different antimicrobials, especially to Bulgecin A (a predictive lytic transglycosylase inhibitor), opening perspectives for new therapeutic approaches.

The investigation of the function of Hp506 in H. pylori would then constitute a good approach to better understand the mechanisms of the morphogenesis of rod-shaped sacculi, still not clearly elucidated.

Abstract no.: W5.3
Motility of H. pylori Is Dependent on luxS-required Production of AI-2 but not its Effect on Sulphur Metabolism

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Introduction: Helicobacter pylori luxS has been shown to function both in biosynthesis of the putative quorum sensing (QS) molecule autoinducer-2 (AI-2) and in the cysteine metabolic pathway with linked metB and cysK (unpublished data). In this study, we show that luxS controls motility of H. pylori via AI-2 signaling, but not via its effect on sulphur metabolism.

Methods: Motility was examined by plate bioassays and light microscopy. Flagellar morphology was studied by the electron microscope. The transcription of flagellar genes was measured using quantitative reverse transcription polymerase chain reaction and flagellar proteins were analyzed by Western blot.

Results: Plate motility assay showed that the wild-type ΔluxS and ΔcysK strains remained motile but the ΔluxS mutant was not. The motility defect of the ΔluxS mutant was restored by genetic complementation and by addition of AI-2, but could not be restored in a cysteine-replete system. Microscopy showed most of wild-type and complemented ΔluxS− cells were swimming, whereas ΔluxS cells were nonswimming. However, the majority of cells in all three strains contain normal flagella. The transcription of some flagellar genes (ΔluxS−) decreased by loss of luxS, consistent with the reduced production of flaA and flgE observed in the ΔluxS mutant.

Conclusions: luxS-dependent production of AI-2, rather than an intact cysteine provision pathway, is required for motility of
**Abstract no.: W5.4**

**DNA Transfer in H. pylori**

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*Helicobacter pylori* colonizes the stomachs of more than 50% of the world’s population, making it one of the most successful human pathogens. Because its genome is subjected to rapid mutation and frequent recombination events, *H. pylori* is one of the most diverse bacterial species. The intraspecies sequence variation is not well understood, but it is known to be associated with natural transformation competence and a conjugation-like mechanism. Indications that conjugative plasmid transfer occurs in *H. pylori* came from the sequence determination of the cryptic plasmid pHel4. Many *H. pylori* strains contain cryptic plasmids and their sequences suggest extensive gene shuffling during evolution and spread of these plasmids. In contrast to other naturally competent bacteria, *H. pylori* uses a type IV secretion system (T4SS), named ComB-T4SS, for DNA uptake. Besides, the ComB-T4SS *H. pylori* strain P12 possesses the Cag-T4SS, the *tfs*3, and a recently by our laboratory identified novel T4SS, named *tfs*4. In this project we are interested in elucidating DNA transfer between *H. pylori* bacteria and to study the underlying mechanism(s). We expect to identify the machinery that mediates DNA transfer. To achieve this we sequentially deleted the genes encoding T4SS in *H. pylori* strain P12. which are possible candidates for DNA transfer. We could demonstrate transfer of chromosomal DNA and for the first time transfer of an indigenous plasmid of *H. pylori*. We could show that *tfs*4 plays a role in plasmid transfer. The data suggest that several diverse mechanisms contribute to horizontal gene transfer of *H. pylori*.

**Abstract no.: W5.5**

**Role of Lytic Transglycosylases in the Motility of H. pylori**

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*Helicobacter pylori* survival in its niche depends on several virulence factors such as its flagella. As flagella have to cross the peptidoglycan (PG) to be externalized, studying the PG metabolism is essential. *H. pylori*’s peptidoglycan is remodeled by, among others, the lytic transglycosylases (TGL) Slt and MltD. We speculated they might be used for the externalization of the flagella.

We inactivated either *slt* or *mltD* in different strains and observed that at least one of the two TGLs is required for full motility depending on the genetic background. This loss of motility of the TGL mutant led to an impaired ability to colonize mice gastric mucosa, despite an identical TGL activity in different strains. Then, we looked for the presence of flagella in mutants affected in motility by scanning electronic microscopy: surprisingly, TGL inactivation did not impair normal flagella assembly. Indeed, we found the same proportion of flagella per bacteria in mutants and their parental strains. Accordingly, flaA expression was identical. Finally, we inactivated the homologous hydrolyase *stlY* in *Escherichia coli* and *Salmonella typhimurium*: this inactivation led to a similar loss of motility.

This study shows for the first time that lytic transglycosylases are involved in the motility of *H. pylori*, but without interfering with the flagella’s assembly. MotB, a flagella motor protein, have been described to have a PG-binding domain. It might be possible that Slt and MltD are involved in the binding of MotB to PG, producing the correct type of mureopeptide ligand for MotB.

**Abstract no.: W5.6**

**Search for H. pylori Bacteriophages**

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Published *Helicobacter pylori* genome sequences lack prophage sequence data, and it has been suggested that *H. pylori* is a bacterium without specific phages due to the harsh human stomach habitat.

There is evidence that viromes may serve as reservoirs for genes, and in marine environments as many as 10^24 genes are transferred by transduction from virus to host each year in the Earth’s oceans. Phages are known to carry and horizontally transfer host genes, which can contribute to the host niche expansion or acquisition of new metabolic pathways. Although *H. pylori* strains present clear evidence of horizontal gene transfer, transduction has yet to be demonstrated in *H. pylori*. The description of *H. pylori* phages is an incomplete topic in the literature, despite their importance and the possibility of using phage therapy to treat antibiotic multidrug-resistant strains. Currently there are only five references of *H. pylori* bacteriophages in the literature. Phage therapy is the therapeutic use of lytic bacteriophages to treat pathogenic bacterial infections. We have developed an *H. pylori* phage screening procedure to test for the presence of integrated prophages and the presence of *H. pylori* phages in wastewater and human feces. We observed clear lysis plaques on a cell layer, and transmission electron microscopy showed bacteriophage-like structures with a diameter approximately of 100 nm without tails, in one strain, and 15 nm filaments, in another, compatible with a filamentous phage.

**Abstract no.: W5.7**

**Assessment of Clarithromycin Resistance in a High-Prevalence Area by Stool PCR Versus Conventional Invasive Testing: Impact on H. pylori Eradication in Children**

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**Background:** In case of an infection with *Helicobacter pylori* resistant to clarithromycin, treatment failure is very likely if a
clarithromycin-based regime is used. The present study was to assess the eradication rate in a region with a high prevalence of clarithromycin resistance in children whose treatment was based on the results of a real-time stool polymerase chain reaction (PCR) allowing for the detection of clarithromycin resistance in comparison to those who were treated according to the results of conventional susceptibility testing.

**Patients and Methods:** Either stool PCR or upper endoscopy followed by culture and susceptibility testing by E-test was conducted in *H. pylori*-infected children presenting with dyspeptic symptoms to an outpatient clinic for pediatric gastroenterology. For eradication, a combination treatment consisting of a proton pump inhibitor, amoxicillin, and either clarithromycin, metronidazole, or levofloxacin was given for 7 to 14 days. Eradication was defined by a negative result of two different noninvasive tests conducted at the earliest 6 weeks after the end of treatment.

**Results:** Seventy-six children were treated between April 2007 and April 2009. In those patients receiving treatment according to resistance testing by stool PCR, eradication was achieved in 33 of 41 (80.5%). In the group whose treatment was tailored according to the results of stool PCR as compared to those whose treatment was tailored based on conventional invasive resistance testing.

**Conclusion:** To E-test results, the eradication rate was 27 of 35 (77.1%).

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**Abstract no.: W6.1**

**Influence of *H. pylori* on the Expression and Activity of Deubiquitinating Enzymes of Human Gastric Epithelial Cells**

N. Lotzing,† R. Sompallae,‡ P. Olbermann,† M. G. Masucci† and C. Josenhans†

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*Helicobacter pylori* is a recognized cancerogenic bacterial agent in humans, which is associated with gastritis, peptic ulcer, and gastric cancer.

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**Abstract no.: W5.8**

**Antimicrobial Resistance of *H. pylori* Isolates in Alaska Native Persons from 2000–2008: Results from the Alaska Sentinel Surveillance Project**

A. Tveit,* M. G. Bruce,* D. Bruden,† J. Morris,† D. Hurlburt† and B. McMahon*†

*Alaska Native Medical Center, Anchorage, Alaska, USA; †Centers for Disease Control and Prevention, Anchorage, Alaska, USA

**Introduction:** *Helicobacter pylori* infection is more common in Alaska Native (AN) people than the general US population, with seroprevalence approaching 75%.

**Methods:** AN persons undergoing endoscopy between January 2000 and December 2008 were identified by the Centers for Disease Control and Prevention’s sentinel surveillance system, and biopsy specimens were cultured to isolate *H. pylori*. Susceptibility testing (agar dilution) for metronidazole [minimum inhibitory concentration (MIC) of ≥ 8 µg metronidazole/mL], clarithromycin (MIC ≥ 1), amoxicillin (MIC ≥ 1), and tetracycline (MIC ≥ 2) was performed on *H. pylori* isolates from 531 persons and levofloxacin testing (MIC ≥ 2) was performed on isolates from 155 persons.

**Results:** *H. pylori* was isolated from 531 of 1180 (45%) persons undergoing upper endoscopy. Metronidazole resistance was demonstrated in isolates from 222 (42%) persons, clarithromycin resistance from 159 (30%) persons, amoxicillin resistance from 10 (2%) persons, and levofloxacin resistance from 30 (19%) persons. No isolates demonstrated tetracycline resistance. Dual resistance to clarithromycin and metronidazole was observed in 15% (82 of 531) of persons. Metronidazole, clarithromycin, and levofloxacin resistance varied by region. Female patients were more likely than male patients to demonstrate metronidazole and clarithromycin resistance (p < .01 both). No statistically significant change in the proportion of persons with resistant isolates was observed over time.

**Conclusion:** Resistance to metronidazole, clarithromycin, and levofloxacin is more common among *H. pylori* isolates from AN persons residing in Alaska compared to those from elsewhere in the United States. Resistance varied by region and no increase in the proportion of resistant isolates was observed over the time period of the study.
clarithromycin-based regime is used. The present study was to assess the eradication rate in a region with a high prevalence of clarithromycin resistance in children whose treatment was based on the results of a real-time stool polymerase chain reaction (PCR) allowing for the detection of clarithromycin resistance in comparison to those who were treated according to the results of conventional susceptibility testing.

Patients and Methods: Either stool PCR or upper endoscopy followed by culture and susceptibility testing by E-test was conducted in H. pylori-infected children presenting with dyspeptic symptoms to an outpatient clinic for pediatric gastroenterology. For eradication, a combination treatment consisting of a proton pump inhibitor, amoxicillin, and either clarithromycin, metronidazole, or levofloxacin was given for 7 to 14 days. Eradication was defined by a negative result of two different noninvasive tests conducted at the earliest 6 weeks after the end of treatment.

Results: Seventy-six children were treated between April 2007 and April 2009. In those patients receiving treatment according to resistance testing by stool PCR, eradication was achieved in 33 of 35 (94.3%). In the group whose treatment was tailored according to the results of stool PCR allowing for the detection of clarithromycin resistance in comparison to those who were treated according to the results of conventional susceptibility testing, eradication was achieved in 33 of 41 (80.5%). In the group whose treatment was tailored according to conventional susceptibility testing, eradication was achieved in 27 of 35 (77.1%).

Conclusion: In an area with a high prevalence of clarithromycin-resistant H. pylori, similar eradication rates were achieved in children treated according to the results of stool PCR as compared to those whose treatment was tailored based on conventional invasive resistance testing.

Abstract no.: W6.1
Influence of H. pylori on the Expression and Activity of Deubiquitinating Enzymes of Human Gastric Epithelial Cells

N. Lotzing, R. Sompallae, P. Olbermann, M. G. Masucci and C. Josenhans

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Helicobacter pylori is a recognized cancerogenic bacterial agent in humans, which is associated with gastritis, peptic ulcer, and gastric cancer.

Abstract no.: W5.8
Antimicrobial Resistance of H. pylori Isolates in Alaska Native Persons from 2000–2008: Results from the Alaska Sentinel Surveillance Project

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Conclusion: Resistance to metronidazole, clarithromycin, and levofloxacin is more common among H. pylori isolates from AN persons residing in Alaska compared to those from elsewhere in the United States. Resistance varied by region and no increase in the proportion of resistant isolates was observed over the time period of the study.

Workshop 6: Gastric Cancer II

Abstract no.: W6.1
Influence of H. pylori on the Expression and Activity of Deubiquitinating Enzymes of Human Gastric Epithelial Cells

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Helicobacter pylori is a recognized cancerogenic bacterial agent in humans, which is associated with gastritis, peptic ulcer, and gastric cancer.

Immunoevasive and immunomodulatory mechanisms underlie the chronic persistence of the bacterium and the active pro-inflammatory effect of life-long H. pylori infection, which, in susceptible hosts, may trigger and drive cancerogenesis. In contrast to tumorigenic viruses, which frequently possess factors to influence the host ubiquitin–proteasome system (UPS) for the purpose of subverting immune responses, nothing is yet known about potential effects of H. pylori in this respect. The majority of H. pylori isolates worldwide possess a pathogenicity island (PAI), cagPAI, which is involved in interleuking (IL)-8 production and chronic inflammation in the context of the infection. We hypothesized that the cagPAI may have an influence on host cell ubiquitin pathways.

The influence of H. pylori wild-type and isogenic mutants lacking the complete cagPAI (or single PAI proteins) on host...
deubiquitinating enzymes was tested in coinubcation experiments with various human gastric epithelial cell lines. Known deubiquitinating enzymes (DUBs) were identified to be active in human gastric cell lines. An influence of the H. pylori infection on the expression and activity of defined cellular UPS proteins, namely the DUB USP7 (HAUSP), was clearly determined. cag-dependent as well as cag-independent effects on the activity and expression of deubiquitinating enzymes were observed in infected cells. These results are a basis for further investigations into potential H. pylori modulators of cellular UPS functions.

Abstract no.: W6.2

H. pylori CagPAI-Mediated Gastritis Induces Later Precancerous Changes in Mongolian Gerbils

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Background: Helicobacter pylori colonization is associated with the development of severe gastroduodenal diseases. Epidemiologic studies have shown that a corpus dominant atrophic gastritis is a risk factor to develop gastric cancer.

Aim: Therefore, we investigated the precancerous process starting with a H. pylori-associated gastritis, followed by an atrophy, metaplasia, dysplasia, and finally leading to a gastric adenocarcinoma using the Mongolian gerbil model.

Methods: The animals (n = 170) were infected with H. pylori B128 (WT) or an isogenic ΔcagY-mutant for 2 to 64 weeks. Paraffin sections of antrum and corpus mucosa were graded and histologic changes were verified by immunohistochemistry. Physiologic and inflammatory markers were measured by radioimmunoassay and quantitative reverse transcription-polymerase chain reaction, respectively.

Results: In comparison to the ΔcagY-mutant, the WT-infected corpus mucosa revealed already a severe atrophic inflammation after 8 weeks, accompanied by a significant up-rise of proinflammatory cytokines such as interleukin 1-β, tumor necrosis factor-α, interferon-γ, and Keratinocyte-derived Chemokine (KC). Physiologic markers (pH, gastrin) and histologic changes of the mucosa towards atrophy, metaplasia, and dysplasia were occurring during late infection. After 14 months of WT infection we found severe histologic changes of the corpus showing typical precancerous markers and prominent gastritis cystica profunda. There was only one WT-infected gerbil developing gastric cancer.

Conclusion: Our data reveal that an intact cagPAI is responsible for an early severe inflammation inducing later physiologic and histopathologic changes. Each step of the cancer pathway could be shown, even up to developing gastric cancer. Therefore, we conclude that the gerbil model is one of the best models mimicking the human situation.

Abstract no.: W6.3

Role of H. pylori Eradication Therapy Success on 5-Year Dynamics of Atrophic Gastritis and Intestinal Metaplasia Grade

T. Filipec Kanizaj, M. Katicic, B. Skurla, M. Prskalo, V. Colic Cvrle, S. Naumovski Mihalic, A. Mrzljak and B. Sabaric

University Hospital Merkur, Zagreb, Croatia

Aim: To investigate the role of eradication therapy success on 5-year dynamics of atrophic gastritis and intestinal metaplasia.

Materials and Methods: The study was performed on 186 Helicobacter pylori-positive patients with atrophic gastritis and/or intestinal metaplasia in basic biopptic specimens analyzed according to updated Sydney protocol. All patients received triple eradication therapy. Therapy success and dynamics of histopathologic parameters were evaluated according to fifth year histologic finding.

Results: Basically 50 patients had atrophic gastritis in corpus and 62 in antrum, 60 intestinal metaplasia in corpus and 116 in antrum mucosa. H. pylori was successfully eradicated in 157 patients. In successfully eradicated, regression/progression/no dynamics in grade of atrophic gastritis revealed 28.66/1.27/70.06% patients in corpus and 33.76/0.64/65.61% in antrum mucosa, respectively. Accordingly, 19.11/3.82/77.07% with intestinal metaplasia in corpus and 41.10/19.19/48.11% in antrum. In noneradicated patients: 13.79/6.90/79.31% with atrophic gastritis in corpus, 20.69/10.34/68.97% atrophic gastritis in antrum, 24.14/6.90/68.97% intestinal metaplasia in corpus, and 31.03/13.79/55.17% in antrum. Statistically significant dynamics of grade of both histologic parameters was observed for successfully H. pylori-eradicated patients only (Wilcoxon rank sum test, p < .001). By multivariate analysis unsuccessful eradication therapy was predictor of progression of grade of atrophic gastritis on both anatomic localizations (antrum p = .014, corpus p < .001).

Conclusions: Successful H. pylori eradication is related with statistically significant dynamics in grade of both histologic parameters. Proportion of patients with regression of atrophic gastritis is statistically significantly higher in successfully H. pylori-eradicated patients than noneradicated. Unsuccessful eradication is predictor of progression of grade of atrophic gastritis on both localizations.

Abstract no.: W6.4

Genetic Variation in the Trefoil Factor (TFF) Genes and Gastric Carcinogenesis

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Trefoil factors (TFFs) play an important role in gastrointestinal mucosal maintenance, and genetic variation in their genes (TFF1, TFF2, and TFF3) could influence the gastric mucosa response to H. pylori infection or to other gastric lesions. The purpose of this study was to characterize variability in the TFF cluster and to
Abstract no.: W6.5
BMP and H. pylori Downregulate SOX2 Expression in Gastric Cell Lines

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Helicobacter pylori colonization of the gastric mucosa is associated with the development of intestinal metaplasia (IM), a preneoplastic lesion characterized by ectopic expression of the intestinal gene CDX2. It was recently demonstrated that H. pylori infection is associated with an increased activity of the BMP/SMAD signaling pathway in gastric epithelial cells and that this pathway positively regulates the expression of CDX2 in the gastric context, via SMAD4. Our results show that, in the gastric carcinoma cell line AGS, BMP-2 increased CDX2 expression and decreased SOX2 expression, as determined by real-time polymerase chain reaction and Western blotting. Additionally, this tendency was reversed when SMAD4 was silenced by RNAi. Upon H. pylori infection, a decrease in SOX2 and an increase in CDX2 expression were observed in AGS and MKN45 cells, independently of the H. pylori cagPAI status. Finally, we verify that SOX2 regulates CDX2 expression, both repressing its promoter and decreasing its expression at the mRNA level. However, SOX2 mRNA expression was not regulated by CDX2 in gastric or in intestinal cell lines.

We conclude that BMP/SMAD and H. pylori infection independently downregulate SOX2, which in turn negatively regulate CDX2, perhaps leading to loss of gastric phenotype and gain of intestinal differentiation.
mucosa after Helicobacter infection. These cells are supposed to follow a mesenchymal to epithelial differentiation in order to regenerate the damaged epithelium. But, due to the infectious and inflammatory environment, this differentiation would be altered and MSC would become tumor-initiating cells.

Our aim is to reproduce in vitro the different steps leading to MSC recruitment and differentiation to identify key factors of carcinoma initiation.

We have already shown that infection of epithelial cells with some H. pylori strains induces MSC migration and invasion. The strains inducing MSC recruitment are those who were the most proapoptotic effective on epithelial cells, suggesting a link between apoptosis and recruitment.

Here, a model of epithelial differentiation of MSC has been developed. MSC and AGS gastric epithelial cells were cultured together or separated by a microporous filter allowing only the traffic of small molecules, and we looked for expression of epithelial markers (epithelial-specific antigen, cytokeratins) on MSC. We showed that MSC can express epithelial markers after fusion with epithelial cells. Transdifferentiation of MSC is also possible when MSC and epithelial cells are cultured separately with a microporous filter.

Effects of H. pylori infection on this differentiation and the evolution of differentiated cells are now being studied, and the results obtained in vitro will be compared with an in vivo model.

**Abstract no.: W6.8**

**Gastric Cancer Occurrence in Preneoplastic Lesions: A Long-Term Follow Up in a High-Risk Area in Spain**


*Catalan Institute of Oncology, Barcelona, Spain; †Complejo Hospitalario de Soria, Soria, Spain; ‡Alcalá University Hospital Príncipe de Asturias, Madrid, Spain

**Aims:** Identification of predictors of gastric cancer (GC) occurrence in gastric preneoplastic lesions.

**Material and Methods:** Prospective-retrospective study based on 478 patients with preneoplastic lesions of nonatrophic gastritis (NAG), nonmetaplastic multifocal atrophic gastritis (MAG), and complete and incomplete intestinal metaplasia (IM), who underwent gastroscopy and biopsy at the beginning and at the end of follow up. Information on Helicobacter pylori infection, family history of GC, smoking, and consumption of nonsteroidal anti-inflammatory drugs (NSAID) were obtained. Inter- and intraobserver variability of histologic diagnosis was assessed. Cox proportional hazards models were used to estimate hazards ratios (HR).

**Results:** Mean age was 50.0 years (47% males), and mean follow up was 12.8 years. During the study term, 23 GC (4.8%) were diagnosed (2 lymphomas and 21 adenocarcinomas). Of the 21 adenocarcinomas, in 16 cases baseline diagnosis was incomplete IM, in 1 case was complete IM, and in 4 cases MAG. The risk of developing GC was higher in males [HR 3.2; 95% confidence interval (CI) 1.1–9.8), older than 60 years (HR 1.7; 95% CI 0.4–6.5), those with family history of GC (HR 5.2; 95% CI 1.4–19.0), and those with incomplete IM (HR 8.8; 95% CI 3.0–25.7). It was lower in those with regular consumption of NSAID.

**Conclusion:** Subtyping of IM, family history of GC, and gender may be useful for identification of high-risk patients.

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**Workshop 7: Treatment**

**Abstract no.: W7.1**

**Sequential Therapy Versus Standard Triple Therapies for H. pylori Infection in Children**

P. Bontems, N. Kalach, G. Oderda, L. Muyschont, A. Salame, Y. Miendje Deyi, S. Cadranel and M. Scaillon

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**Background:** Assess the eradication rate of Helicobacter pylori in children using a sequential regimen compared with the classical triple therapy.

**Results:** From October 2007 to February 2009, 125 children were included (75 female/50 male, median age 11 years, range 1.5 to 17). Eradication achieved in 92 children out of 110 who returned for a follow-up test. The intention-to-treat (ITT) eradication rate was thus 74% (sequential 51 of 66 = 77%, triple therapy 41 of 59 = 69%) and the per-protocol (PP) cure rate 84% (sequential 51 of 60 = 85%, triple therapy 41 of 50 = 82%). In case of CLA resistance, ITT eradication rate was 10 of 18 (sequential 7 of 14 = 50%, triple therapy 3 of 4) and PP 10 of 16 (sequential 7 of 12 = 58%, triple therapy 3 of 4). In case of MET
mucosa after Helicobacter infection. These cells are supposed to follow a mesenchymal to epithelial differentiation in order to regenerate the damaged epithelium. But, due to the infectious and inflammatory environment, this differentiation would be altered and MSC would become tumor-initiating cells.

Our aim is to reproduce in vitro the different steps leading to MSC recruitment and differentiation to identify key factors of carcinoma initiation.

We have already shown that infection of epithelial cells with some H. pylori strains induces MSC migration and invasion. The strains inducing MSC recruitment are those who were the most proapoptotic effective on epithelial cells, suggesting a link between apoptosis and recruitment.

Here, a model of epithelial differentiation of MSC has been developed. MSC and AGS gastric epithelial cells were cultured together or separated by a microporous filter allowing only the traffic of small molecules, and we looked for expression of epithelial markers (epithelial-specific antigen, cytokeratins) on MSC. We showed that MSC can express epithelial markers after fusion with epithelial cells. Transdifferentiation of MSC is also possible when MSC and epithelial cells are cultured separately with a microporous filter.

Effects of H. pylori infection on this differentiation and the evolution of differentiated cells are now being studied, and the results obtained in vitro will be compared with an in vivo model.

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Gastric Cancer Occurrence in Preneoplastic Lesions: A Long-Term Follow Up in a High-Risk Area in Spain

C. A. Gonzalez,* J. Ruiz,† P. Alonso,‡ M. L. Pardo,‡ C. Bonet,* F. Marin,* G. Capella,* N. Sala* and J. Sanz-Anguera†
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Aims: Identification of predictors of gastric cancer (GC) occurrence in gastric preneoplastic lesions.

Material and Methods: Prospective-retrospective study based on 478 patients with preneoplastic lesions of nonatrophic gastritis (NAG), nonmetaplastic multifocal atrophic gastritis (MAG), and complete and incomplete intestinal metaplasia (IM), who underwent gastroscopy and biopsy at the beginning and at the end of follow up. Information on Helicobacter pylori infection, family history of GC, smoking, and consumption of nonsteroidal anti-inflammatory drugs (NSAID) were obtained. Inter- and intraobserver variability of histologic diagnosis was assessed. Cox proportional hazards models were used to estimate hazards ratios (HR).

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Background: Assess the eradication rate of Helicobacter pylori in children using a sequential regimen compared with the classical triple therapy.

Methods: Prospective, open-label, multicenter study. Children received randomly either a 10-day sequential treatment comprising omeprazole with amoxicillin (AMO) 5 days and omeprazole, clarithromycin (CLA) and metronidazole (MET) 5 days or a 7-day treatment comprising omeprazole with AMO and CLA (or MET in case of resistance to CLA). Eradication was assessed by 13C-urea breath test.

Results: From October 2007 to February 2009, 125 children were included (75 female/50 male, median age 11 years, range 1.5 to 17). Eradication achieved in 92 children out of 110 who returned for a follow-up test. The intention-to-treat (ITT) eradication rate was thus 74% (sequential 51 of 66 = 77%, triple therapy 41 of 59 = 69%) and the per-protocol (PP) cure rate 84% (sequential 51 of 60 = 85%, triple therapy 41 of 50 = 82%). In case of CLA resistance, ITT eradication rate was 10 of 18 (sequential 7 of 14 = 50%, triple therapy 3 of 4) and PP 10 of 16 (sequential 7 of 12 = 58%, triple therapy 3 of 4). In case of MET
resistance, ITT eradication rate was 20 of 29 (sequential 11 of 17 = 65%, triple therapy 9 of 12 = 75%) and PP 20 of 26 (sequential 11 of 14 = 79%, triple therapy 9 of 12 = 75%).

**Conclusion:** Sequential treatment seems highly effective, with similar or higher eradication rate than with a triple therapy prescribed in accordance with antimicrobial susceptibility testing. However, in case of CLA or MET resistance the cure rate is decreased.

**Abstract no.: W7.2**

Detection of *H. pylori* and Clarithromycin Resistance in Biopsies from Pediatric Patients by New Commercial Kit Genotype® HelicoDR

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Clarithromycin susceptibility is an important factor to predict *Helicobacter pylori* eradication failure. In addition, the resistance to this antibiotic is high in Spain. Therefore, early detection of resistance is important in treatment of *H. pylori* infection. The aim of this study was to evaluate a commercially available kit Genotype® HelicoDR (Hain, Diagnostika, Nehren, Germany) for detection of *H. pylori* strains that are clarithromycin resistant and compare it with conventional protocols.

A total of 63 biopsies were obtained from patients with gastritis symptoms. Standard procedures were used for *H. pylori* culture. Clarithromycin resistance was determined by E-test as described before. DNA extraction was carried out by the NucliSens easyMAG platform (bioMérieux), and Genotype® HelicoDR was used to assess clarithromycin susceptibility as well as *vacA*, *cagA*, and *cagA* PCR were performed to confirm the results with Genotype®.

We confirmed that 44% of patients were *H. pylori* negative by all the methods. Among those positive for *H. pylori* 24 (68.5%) of 35 were positive by culture. Clarithromycin resistance was observed in 45.8% of the strains by E-test, in 45.8% of the strains by Genotype® and in 40.8% of the strains by conventional molecular methods. Among the 11 negative cultures but positive by Genotype®, six were negative by other methods but five (45.4%) were confirmed to be *H. pylori* positive by molecular techniques, and we detected two *H. pylori* strains that are clarithromycin resistant.

We confirmed the high prevalence of clarithromycin-resistant strains among patients from Spain, and the use of a commercial kit is as reliable as conventional methods.

**Abstract no.: W7.3**

Multicenter Study on the Treatment of *H. pylori* Infection with High Dose Esomeprazole, Amoxicillin, and Metronidazole in Children Infected with Double-Resistant Strains

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**Background:** The increasing number of pediatric patients with multiresistant *Helicobacter pylori* strains creates urgent need for evaluation of treatment regimens. Second-line antibiotics like tetracycline, chinolons, or bismuth are not released for children. However, in vitro resistance to metronidazole may be overcome by a high dose and prolonged intake.

**Objective:** Prospective multicenter study on eradication rate and side-effects of a high dose triple therapy in pediatric patients with culture-proven double resistance.

**Methods:** In this investigator-initiated open treatment trial, including several European countries, 58 *H. pylori*-infected patients (15 kg) with proven resistance to metronidazole and clarithromycin were prospectively included. Therapy including amoxicillin (∼75 mg/kg), metronidazole (∼25 mg/kg), and esomeprazole (∼1.5 mg/kg) was given twice daily for 2 weeks. Success of therapy was monitored by a 13C-urea breath test at 6 and 24 weeks after treatment. Primary outcome parameter was the eradication rate at 6 weeks.

**Results:** Of 58 children included, follow-up data were available until May 2009 from 50 patients after 6 weeks and from 34 after 24 weeks follow up. Eradication rates after 6 weeks were 81% (per protocol) and 66% (intention to treat analysis), respectively; no serious side-effect occurred.

**Conclusions:** A high dose 2-week therapy with amoxicillin, metronidazole, and esomeprazole is a well-tolerated treatment option in children infected with a double-resistant *H. pylori* strain. Eradication rate is good when drugs were taken according to the protocol. However, compliance in this patient group was difficult to achieve.

**Abstract no.: W7.4**

Omega 3 Docosahexaenoic Acid Inhibits *H. pylori* Gastric Colonization in C57BL/6 Mice

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*Helicobacter pylori* infection is recognized as the major etiologic factor in gastric cancer. Drug-resistant strains of *H. pylori* and noncompliance to therapy are the major causes of treatment failure. For some bacterial species it has been demonstrated that certain lipids have a growth inhibitory effect. In order to...
Abstract no.: W7.5

Effect of Eradication of H. pylori on Maximum Tolerated Volume and Dyspeptic Symptoms in Patients with Functional Dyspepsia

Aga Khan University, Karachi, Pakistan; Karolinska Institute, Stockholm, Sweden

Objectives: To compare maximum tolerated volume (MTV) in patients with or without Helicobacter pylori infection and to study the effect of eradication of H. pylori on MTV and dyspeptic symptoms.

Materials and Methods: Patients with FD were divided into H. pylori-positive and -negative groups. Symptoms and demographics were recorded. MTV was determined by satiety drink test using slow drinking (30 mL/min) of a nutrient liquid. H. pylori-positive patients received 1 week eradication therapy and additional proton pump inhibitors (PPI) for 3 weeks. H. pylori-negative group received PPI for 4 weeks. MTV, dyspeptic symptoms, and H. pylori status were rechecked.

Results: A total of 128 patients, mean age 38 ± 11 years (18–65), were evaluated. H. pylori were present in 59 (46%). MTV of H. pylori-negative and -positive patients were 393 ± 202 mL vs 381 ± 237 mL, respectively (p = not significant). Eradication of H. pylori was successful in 50 of 59 (85%) patients. Increase in MTV after therapy was noted in H. pylori-negative patients and H. pylori (successfully eradicated) patients, 56 ± 77 mL and 188 ± 102 mL (p < .01), respectively, but not in patients with unsuccessful eradication.

Multiple regression analysis showed that age, weight, and H. pylori-positive status have a significant impact on MTV. Overall dyspeptic symptoms improved significantly in both groups; however, bothersome symptoms were improved more in H. pylori (successfully eradicated) patients.

Conclusion: Eradication of H. pylori was associated with significant improvement in maximum tolerated volume and dyspeptic symptoms. Estimation of maximum tolerated volume may be useful for assessment beside symptoms in patients with functional dyspepsia.

Abstract no.: W7.6

Analysis of Related Factors of H. pylori Eradication Therapy Efficiency

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Gastrointestinal Department, Beijing University Third Hospital, Beijing, China

Objective: To investigate the related factors of Helicobacter pylori eradication.

Materials and Methods: A total number of 1034 patients diagnosed as H. pylori infected by Warthin-Starry (WS) stain were enrolled and received H. pylori eradication therapy. The 14C-urea breath test was taken more than 4 weeks after the therapy. Then the related factors for H. pylori eradication was investigated.

Results: H. pylori eradication rate was 75.0%. The eradication rate of the peptic ulcer group was higher than the nonulcer group (87.3% and 71.4%, p = .000). The eradication rate of the initial treated group was 75.7%, higher than that of the re-treated group (64.5%, p = .048). The eradication rate of the 14-day and the 7-day groups were 92.1% and 74.8% (p = .002), respectively. The eradication rate of proton pump inhibitor (PPI)-based group was higher than the non-PPI-based group (80% and 62.6%, p = .000). The eradication rate of the fluoroquinolone group (67.4%) was significantly lower than that of the classic antibiotics group (76.5%). The eradication rate of PPI-based triple therapy (77.6%) was higher than the non-PPI-based therapy (63.4%). The eradication rate of the fluoroquinolone group (58.9%) was significantly lower than that of the classic antibiotics group (73.0%). In the PPI- and bismuth-based quadruple regimen, the eradication rate of the amoxicillin plus clarithromycin group was the most effective (eradication rate 92.3%).

Conclusions: Quadruple, 14-day, PPI-based therapy was effective for H. pylori eradication. Amoxicillin plus clarithromycin with PPI- and bismuth-ased quadruple therapy was the most effective regimen for H. pylori eradication.

Abstract no.: W7.7

Probiotics Exerted Anti-inflammatory Activities through SOCS-2/SOCS-3 Expression via Simultaneous STAT-1/STAT-3 Activation and JAK2 Inactivation Against H. pylori Infection

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Gachon University of Medicine and Science, Incheon, South Korea; Mediogen Biotechnology, Seoul, South Korea

In spite of IARC definition that Helicobacter pylori is the definite carcinogen of gastric cancer, the simple removal of bug is not sufficient for preventing gastric cancer and increasing microbial resistance limits the eradication application. Therefore, probiotics, nonpathogenic microbial feed that can affect the host in a beneficial manner, could be an alternate way for enhancing anti-inflammation. However, the mechanism how probiotics can impose anti-inflammatory actions against H. pylori is still not documented clearly. In the current study, we hypothesized that suppressor of cytokine signaling (SOCS) by probiotics could be a potential anti-inflammatory mechanism against H. pylori infection. H. pylori infection or their lipopolysaccharide stimulation led to significant increased expressions of inflammatory mediators including TNF-α, IL-8, iNOS, and COX-2 in AGS cells and coadministration of L. plantarum, L. rhamnosis, and L. acidophilus significantly attenuated expressions of these inflammatory mediators.
mediators in accordance with blocking action of NF-κB nuclear translocation. Probiotics administration induced SOCS-2 and -3 expressions and probiotics could exert the activation of SOCS signaling featuring earlier and higher expressions of SOCS-2 and SOCS-3. In contrast to weak inactivation of MAPKs including p-38 and ERK1/2, probiotics phosphorylated STAT-1 and STAT-3 significantly and provoked simultaneous inhibition of JAK2 phosphorylation, which is related to negative signaling of SOCS-2/-3. Taken together, anti-inflammatory signals through STAT activation and JAK2 inactivation might be fundamental mechanism of probiotics against *H. pylori* infection, rendering probiotics nonmicrobial approach to *H. pylori* infection.

**Abstract no.: W7.8**

**Histopathologic and Morphologic Evaluations of Resistant Strains of *H. pylori* and Consequent New Therapeutic Approaches: Usefulness of N-Acetil-Cysteine and Culture-Guided Antibiotics**

G. Cammarota,* G. Ianiro,* A. C. Piscaglia,* A. Cazzato,* F. Ardito,* G. Branca,† R. Torelli,† G. Fadda,* A. Gasbarrini* and G. Gasbarrini*‡

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**Background:** Resistant strains of *Helicobacter pylori* can display a dense biofilm with mucus and microorganisms in a coccoid shape on the mucosal surface of stomach that may have a role in determining the resistance to the antibiotic therapies. Possibly, N-acetil-cysteine (NAC) may dissolve biofilm architecture and help to eradicate resistant strains of *H. pylori*. 

**Aim:** To evaluate the usefulness of NAC as pre-treatment attempt associated with a culture-guided antibiotic therapy as rescue therapy after multiattempts antibiotic failure.

**Methods:** Thirty-one patients, after at least two antibiotic unsuccessful eradication attempts for *H. pylori*, were consecutively recruited. In all patients antibiotic susceptibility testing was performed. Group A patients received NCA 600 mg once a day for a week and subsequently a culture-guided 1-week regimen including a proton pump inhibitor (PPI) plus two antibiotics; group B patients received solely a culture-guided 1-week antibiotic treatment including a PPI plus two antibiotics. Sensitive antibiotics were always chosen on the basis of the more favorable minimum inhibiting concentration value. Patients took a control C13 urea breath test 2 months after the end of therapy.

**Results:** Ten of 16 patients (62%) in group A returned *H. pylori* negative at the follow up, while only four of 15 patients (27%) in group B were successfully treated.

**Conclusions:** This pilot study shows that NAC pre-treatment may be useful in eradicating *H. pylori* when associated to a culture-guided 1-week antibiotic rescue regimen. Possibly, NAC could dissolve the mucus biofilm making *H. pylori* strains vulnerable to the antibiotic attack. Further larger studies are needed to confirm our preliminary results.

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**Workshop 8: Genetics and Virulence Factors**

**Abstract no.: W8.1**

**The Complete Genome Sequence of *H. pylori* Strain P12: Insights into Gene Transfer and Genome Evolution**

W. Fischer,* L. Windhager,† S. Rohrer,* A. Karnholz,* M. Zeiller,* R. Hoffmann,‡ R. Zimmer† and R. Haas§

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Many bacteria exhibit a surprising extent of intraspecies genome variability, with the core genomes often representing only a small fraction of the entire gene repertoire. *Helicobacter pylori* is known as a species with an enormous sequence diversity in housekeeping genes, and previous data suggest that a considerable variability exists in gene content as well. Genomic comparison of several strains is therefore required to obtain a full view of the host adaptation capabilities of *H. pylori*. Here, we present the complete genome sequence of the duodenal ulcer strain P12.

With a chromosome of 1,673,813 bp and an additional 10,225 bp plasmid, P12 possesses the most comprehensive *H. pylori* genome so far. A comparison with other complete *H. pylori* genome sequences revealed that two individual *H. pylori* strains may differ in up to 12% of their gene content, and the amount of strain-specific genes indicates that *H. pylori* possesses an open pan-genome. Strain-specific genes are often located at potential genome rearrangement sites, but most of them are clustered in three distinct plasticity regions. Two of these plasticity zones are integrated into restriction/ modification genes and are likely to represent genomic islands. Moreover, both encode complete type IV secretion systems, which are related but clearly distinct. Microarray analysis was used to determine the distribution of these type IV secretion systems in different *H. pylori* strains, and mutational analysis indicated their involvement in horizontal gene transfer. Together with the ComB and Cag systems, *H. pylori* P12 contains thus four distinct type IV secretion systems.
mediators in accordance with blocking action of NF-κB nuclear translocation. Probiotics administration induced SOCS-2 and -3 expressions and probiotics could exert the activation of SOCS signaling featuring earlier and higher expressions of SOCS-2 and SOCS-3. In contrast to weak inactivation of MAPKs including p-38 and ERK1/2, probiotics phosphorylated STAT-1 and STAT-3 significantly and provoked simultaneous inhibition of JAK2 phosphorylation, which is related to negative signaling of SOCS-2/-3. Taken together, anti-inflammatory signals through STAT activation and JAK2 inactivation might be a fundamental mechanism of probiotics against *H. pylori* infection, rendering probiotics nonmicrobial approach to *H. pylori* infection.

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**Abstract no.: W8.2**

*H. pylori* Genome Sequencing Using the Roche FLX Titanium Platform; Identification of MALT Strain-Specific Markers

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The overall aim of this project is to sequence the genome of six* Helicobacter pylori* strains (three *cag*PAI (+) and three *cag*PAI (−)) isolated from gastric MALT lymphoma patients using a MID tagging strategy and the Roche FLX Titanium platform. The following data focus on one of the genomes.

The B49 strain was isolated in France from a 29-year-old male suffering from gastric MALT lymphoma. It is *cag*PAI (−), *vacA* s2m2, and Lewis Y (+). A total of 167,745 reads were obtained. Using the newbler assembler, we obtained 48 contigs consisting of 1,626,891 bp. The AMIgene webtool predicted 1657 CDSs which were compared to six genomes available in the Pyloriscope database (MAGE: http://www.genoscope.cns.fr/aage/mage/). A total of 1374, 1330, 1360, 1386, 1420, and 1396 CDSs were shared between B49 and the following six strains: 26,695, J99, HPAG1, 1374, 1350, 1360, 1386, 1420, and 1396 CDSs were shared. We show that the Roche FLX Titanium platform is well suited for *H. pylori* genome sequencing. Our long-term goal is to define the *H. pylori* core genome and metagenome and to identify strain-specific markers of MALT strains.

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**Abstract no.: W8.3**

*The H. pylori* GroES Co-chaperonin, HsPA, Functions as a Specialized Nickel-chaperone and Storage Protein Through its Unique C-terminal Extension

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The transition metal nickel is essential for* Helicobacter pylori* because it is a cofactor for two enzymes indispensable for colonization, urease and [Ni-Fe] hydrogenase. In order to provide these proteins with their cofactor, nickel is scavenged through the outer membrane by FrpB4, a TonB-dependent transporter (Schauer *et al.* 2007) and subsequently imported by an inner membrane permease, NixA. Nickel trafficking in *H. pylori* is original and complex with specific factors for nickel incorporation into these metalloproteins and for nickel storage to protect against toxicity.

The GroES co-chaperonin homologue of *H. pylori*, Hspa, is an essential protein that contains a unique His-rich C-terminal extension demonstrated to bind nickel in vitro. To investigate the function of this extension in *H. pylori*, we constructed mutants carrying a complete deletion or point mutations in this domain. The mutants presented decreased intracellular nickel content (measured by ICP-MS) and reduced nickel tolerance. Sensitivity to bismuth (binding this extension in vitro) was unaltered in the mutants, suggesting that HspA is not the essential target of bismuth-based *H. pylori* eradication therapy. While urease activity was unaffected in the mutants, [Ni-Fe] hydrogenase was significantly diminished when the extension was mutated.

We concluded that the C-terminal domain of Hspa is involved in intracellular nickel storage and detoxification, and in addition, plays a role as a specialized nickel chaperone to achieve hydrogenase maturation. Based on these results, a model for the controlled nickel distribution between urease and hydrogenase in *H. pylori* is proposed.

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**Abstract no.: W8.4**

Genomic Plasticity Region Proteins as Players in Chronic Persistence of *H. pylori*

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*Helicobacter pylori* is a chronic pathogen that has coevolved with its human host. We explored population structure of *H. pylori* in India (and elsewhere) and found that the Indian strains share genetic origins as well as their trajectory of virulence genes with the western strains. Also, it was found that genotypes of some of the chief virulence factors (*CagA* and *VacA*) do not always correlate with particular outcomes of infection. To understand this dilemma we hypothesized a complex interplay of many different virulence factors. We focused initially on a few of the functionally unknown members of the genomic plasticity region, a putative type IV secretion cluster believed to be acquired horizontally. Two members of this cluster (JHP940 and HP986) potentially appeared to be interacting with the human immune system. The HP986 upon computational modeling was putatively identified as a signaling protein and was experimentally proved to be a proinflammatory and proapoptotic agent. Most persistent microbes seemingly evolve strategies to avenge innate responses to gain niche and to maintain growth fitness. For example, *H. pylori* traditionally harnesses its cardinal virulence factors to downregulate T-cell responses (through the VacA-mediated cell cycle arrest) and upregulates mucosal proinflammatory pathways (by CagA). Surprisingly in our studies, HP986 appears to be able to perform both the immune stimulatory and immune evasion tasks single handedly. Thus we believe that HP986 probably functions as a persistence factor which awaits validation using appropriate animal models.
Abstract no.: W8.5

**H. pylori VacA Induces GSK3 Inhibition Through the PI3K/Akt Signaling Pathway**

T. Hirayama,* M. Nakayama,† H. Isomoto,* M. Hatakeyama,* T. Azuma,* Y. Yamaoka* and J. Moss**

* Nagasaki University, Nagasaki, Japan; † Hokkaido University, Sapporo, Japan; ‡ Kobe University School of Medicine, Kobe, Japan; § Oita University School of Medicine and Baylor College of Medicine, Oita and Huston, Japan; ** NHLBI, NIH, Bethesda, Maryland, USA

Vacuolating cytotoxin (VacA) produced by *Helicobacter pylori* contributes to the pathogenesis and severity of gastric injury. We found that incubation of AZ-521 cells with VacA resulted in phosphorylation of protein kinase B (Akt) and glycogen synthase kinase-3β (GSK3β) through a PI3K-dependent pathway. Following phosphorylation and inhibition of GSK3β, β-catenin was released from a GSK3β/β-catenin complex, with subsequent nuclear translocation. Methyl-β-cyclodextrin (MCD) and phosphatidylinositol-specific phospholipase C (PI-PLC), but not 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB) and bafilomycin A1, inhibited VacA-induced phosphorylation of Akt, indicating that it does not require VacA internalization and is independent of vacuolation. VacA treatment of AZ-521 cells transfected with TOPtkLuciferase reporter plasmid or control FOPtkLucifease reporter plasmid resulted in activation of TOPtkLuciferase, but not FOPtkLucifease. In addition, VacA transactivated the β-catenin-dependent cyclin D1 promoter in a luciferase reporter assay. Infection of AZ-521 cells by a VacA mutant strain of *H. pylori* failed to induce phosphorylation of Akt and GSK3β, or release of β-catenin from a GSK3β/β-catenin complex. Taken together, these results support the conclusion that VacA activates the PI3K/Akt signaling pathway, resulting in phosphorylation and inhibition of GSK3β, and subsequent translocation of β-catenin to the nucleus, consistent with effects of VacA on β-catenin-regulated transcriptional activity. These data introduce the possibility that Wnt-dependent signaling might play a role in the pathogenesis of *H. pylori* infection, including the development of gastric cancer.

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Abstract no.: W8.6

**AGS Cells Expressing E-cadherin Establish Tight Junctions and Constitute a Useful Model for Studying *H. pylori* Infection**

A. C. Costa,*† M. L. Pinto,* A. M. Costa,* M. Leite,* R. M. Ferreira,*† M. J. Oliveira,† J. C. Atherton,§ M. Mareel† and C. Figueiredo*†

* IPATIMUP – Institute of Molecular Pathology and Immunology of the University of Porto, Porto, Portugal; † Faculty of Medicine University of Porto, Porto, Portugal; § NDDC BRU – Nottingham Digestive Diseases Centre Biomedical Research Unit, University of Nottingham, Nottingham, UK; ‡ Laboratory of Experimental Cancer Research, Ghent University, Ghent, Belgium

Alterations in the epithelial apical–junctional complex are important in the pathogenesis of *Helicobacter pylori*-associated gastric disease. A major limitation for in vitro studies of the gastric epithelium is the fact that the majority of gastric cell lines deposited in biobanks do not establish an apical–junctional complex and do not form cohesive monolayers. Furthermore, freshly isolated human gastric epithelial cells do not proliferate in primary cultures, restricting their use as an infection model.

Evidence that *H. pylori* affects tight junction integrity comes mainly from human intestinal and canine kidney cell line models. The human gastric AGS cell line is widely used for studying *H. pylori* interactions with the host. However, these cells lack E-cadherin expression and consequently do not form adherens or tight junctions.

AGS cells were stably transduced with E-cadherin (AGSEcad). The expression and cellular localization of proteins of the adherens and tight junctions were evaluated by immunocytochemistry. Junction functionality was evaluated by aggregation, transepithelial electrical resistance, and hydrophilic tracer permeability assays.

AGSEcad cells displayed adherens junction components E-cadherin, β-, α-, and pl 20-catenins and tight junction components occludin, claudins, JAM-A, and ZO-1 at the cell membrane. Furthermore, AGSEcad cells exhibited functional adherens and tight junctions.

Infection of AGSEcad cells with *H. pylori* led to alterations in membrane localization of tight junctional proteins, as well as to significant changes in transepithelial electrical resistance and in dextran permeability, suggestive of decreased barrier function.

In conclusion, AGSEcad cells establish adherens and tight junctions and constitute a useful model for studying *H. pylori* infection.

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Abstract no.: W8.7

**The Number of *H. pylori* CagA EPIYA C Tyrosine Phosphorylation Motifs Is Associated with Histologic Features of Chronic Gastritis**

R. M. Ferreira,*† J. C. Machado,*† F. Carneiro† and C. Figueiredo†

* IPATIMUP – Institute of Molecular Pathology and Immunology of the University of Porto, Porto, Portugal; † Faculty of Medicine University of Porto, Porto, Portugal

Background and Aims: *H. pylori* strains containing CagA are associated with increased gastric carcinoma risk. CagA can be tyrosine phosphorylated within EPIYA motifs, classified as EPIYA-A, EPIYA-B, and EPIYA-C in Western strains. The number of EPIYA-C motifs influences the level of CagA phosphorylation, the degree of SHP-2 binding, and the magnitude of cytoskeleton changes induced by *H. pylori*.

Our aim was to characterize *H. pylori* CagA EPIYA motifs in strains infecting Portuguese patients in order to explore their relationship with the histopathologic features of chronic gastritis.

Materials and Methods: One hundred and seventeen *H. pylori*-infected patients with chronic gastritis were studied. Histopathologic parameters were scored according to the updated Sydney system. The presence of cagA and the number and type of EPIYA motifs were determined by polymerase chain reaction.

Results: Infection with strains with ≥2 CagA EPIYA-C motifs was associated with more severe chronic inflammation in the corpus, and higher grade of polymorphonuclear activity and presence of epithelial damage in both corpus and antrum. The magnitude of risk for gastric atrophy/intestinal metaplasia increased with increasing number of EPIYA-C motifs: the odds ratio was 5.4
(95% confidence interval, 1.6–18) in individuals infected with strains < 2 EPIYA-C motifs, and was 12.1 (95% confidence interval, 2.5–57) in individuals infected with strains with ≥2 EPIYA-C motifs.

**Conclusions:** *H. pylori* strains with ≥2 CagA EPIYA-C motifs are associated with more severe histopathologic features of gastritis and with gastric atrophy/intestinal metaplasia. Characterization of the CagA EPIYA-containing region may be important for a better definition of gastric carcinoma risk.

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**Abstract no.: W8.8**  
**Intestinal Metaplasia of the Gastric Cardia is More Frequent in GERD Patients with Family History of GERD**

NIMTS General, Athens, Greece

**Aim:** To define the effect of family history of gastroesophageal reflux disease (GERD) (FH) on the severity of carditis and intestinal metaplasia of the gastric cardia in GERD patients.

**Patients and Methods:** One hundred and twenty patients with FH(–) GERD (mean age 60 ± 15 years, 73 male) and 120 FH(+) GERD patients (mean age 59 ± 15, 72 male) after gastroscopy with biopsies started on omeprazole 20 mg twice a day for 1 year plus 10-day *H. pylori* eradication regimen if HP(+). Finishing treatment, we repeated endoscopy with biopsies, on omeprazole and 13C-urea breath test, off omeprazole if HP(+). The Sydney classification system was used for carditis/intestinal metaplasia. Stat:X2.

**Results:** Cardiac mucosa was identified in 111 (93%) FH(+), 109 (91%) FH(–) patients (*p = .64*); carditis in 81 (68%) FH(+) and 71 (59%) FH(–) patients (*p = .18*), not correlated with *H. pylori* infection. Intestinal metaplasia of gastric cardia was found in 50 (42%) FH(+), 34 (28%) FH(–) patients (*p = .0001*) [HP(+):23 (19%) FH(+), 11 (9%) FH(–) patients (*p = .007*); HP(–):27 (23%) FH(+), 23 (19%) FH(–) patients (*p = .01*). Thirty-two FH(+) and 29 FH(–) patients eradicated *H. pylori*. Of them 13 FH(+) and 18 FH(–) patients present no carditis in follow-up endoscopy. Seventy (58%) FH(+) and 53 (44%) FH(–) patients presented carditis (*p < .001*). During follow up the severity of intestinal metaplasia increased by 0.4 ± 0.1 grades in FH(+) patients while remained unchanged in FH(–) patients (*p < .001*). Five (4%) FH(+) and no FH(–) patients developed low-grade dysplasia (*p = .02*). Carditis regression was more frequent after *H. pylori* eradication.

**Conclusions:** 1. Intestinal metaplasia of the cardia, but not carditis is more frequent in GERD FH(+) patients. 2. Carditis regresses less frequently after *H. pylori* eradication in FH(+) GERD patients. 3. Intestinal metaplasia of the cardia worsens and dysplasia develops more rapidly in FH(+) GERD patients despite high-dose omeprazole treatment.

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**P01 Diagnosis**

**Abstract no.: P01.01**  
**Pepsinogen I Levels and *H. pylori* in Siberian Populations**

O. V. Reshetnikov,* S. A. Kurilovich,* S. A. Krotov† and V. A. Krotova†  
*Institute of Internal Medicine, Novosibirsk, Russia; †Joint-Stock Company Vector-Best, Novosibirsk, Russia

**Background:** Low serologic pepsinogen I (PGI) value is a reliable surrogate marker of atrophic gastritis. The relationship between atrophic gastritis and *Helicobacter pylori* infection in Russia is still uncertain because of various climatic, socioeconomic, and ethnic differences.

**Aim:** To detect atrophic gastritis and *H. pylori* infection in four Siberian populations using serum biomarker tests.

**Material and Methods:** Representative samples of four populations aged 45–80 years living in Siberia were investigated: 1, urban Caucasians in Novosibirsk, Western Siberia (*n = 264*); 2, urban Caucasians in Yakutsk, Eastern Siberia (*n = 81*); 3, urban Asians in Yakutsk, Eastern Siberia (*n = 72*); and 4, rural Asians in Yakutsk Region, Eastern Siberia (*n = 90*).

Sera were tested for PGI using ELISA (Gastropanel, Biohit). Atrophic gastritis was defined if PGI level was < 25 ng/mL. Anti-*H. pylori* IgG CagA antibodies were evaluated with domestic ELISA kits (Joint-Stock Company Vector-Best, Novosibirsk, Russia).

**Results:** The prevalence of atrophic gastritis was the lowest in urban Caucasians in Novosibirsk and significantly higher in Asian populations (*p = .001*). In contrast, the prevalence of *H. pylori* CagA infection was similar in three populations, being significantly higher in rural Asians (*p = .03*).

**Conclusion:** The prevalence of atrophic gastritis in Caucasians in Russia is higher than in European countries, in Asians being higher than in Japan. The association of atrophic gastritis and CagA positivity differs among populations and needs further investigation.
Material and Methods: Siberian populations using serum biomarker tests. Asians in Yakutsk, Eastern Siberia (n = 72); and rural Asians in 2, urban Caucasians in Yakutsk, Eastern Siberia (n = 81); 3, urban Caucasians in Novosibirsk, Western Siberia (n = 264); 1, urban Caucasians in Novosibirsk, Western Siberia (n = 264); 3, urban Caucasians in Yakutsk, Eastern Siberia (n = 81); 3, urban Caucasians in Yakutsk, Eastern Siberia (n = 81); and 4, rural Asians in Yakutsk Region, Eastern Siberia (n = 90).

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Conclusions: H. pylori strains with ≥ 2 CagA EPIYA-C motifs are associated with more severe histopathologic features of gastritis and with gastric atrophy/intestinal metaplasia. Characterization of the CagA EPIYA-containing region may be important for a better definition of gastric carcinoma risk.

Abstract no.: W8.8
Intestinal Metaplasia of the Gastric Cardia is More Frequent in GERD Patients with Family History of GERD
NIMTS General, Athens, Greece

Aim: To define the effect of family history of gastroesophageal reflux disease (GERD)(FH) on the severity of carditis and intestinal metaplasia of the gastric cardia in GERD patients.

Patients and Methods: One hundred and twenty patients with FH(–) GERD (mean age 60 ± 15 years, 73 male) and 120 FH(+) patients (mean age 59 ± 15, 72 male) after gastroscopy with biopsies started on omeprazole 20 mg twice a day for 1 year plus 10-day H. pylori eradication regimen if HP(+). Finishing treatment, we repeated endoscopy with biopsies, on omeprazole and 14C-urea breath test, off omeprazole if HP(+). The Sydney classification system was used for carditis/intestinal metaplasia. Stat:X2.

Results: Cardiac mucosa was identified in 111 (93%) FH(+), 109 (91%) FH(–) patients (p = .64); carditis in 81 (68%) FH(+) and 71 (59%) FH(–) patients (p = .18), not correlated with H. pylori. Intestinal metaplasia of gastric cardia was found in 50 (42%) FH(+), 34 (28%) FH(–) patients (p = .0001) [FH(+):23 (19%) FH(–):11 (9%) FH(–) patients (p = .007); FH(–):27 (23%) FH(+), 23 (19%) FH(–) patients (p = .01)]. Thirty-two FH(+) and 29 FH(–) patients eradicated H. pylori. Of them 13 FH(+) and 18 FH(–) patients present no carditis in follow-up endoscopy. Seventy (58%) FH(+) and 53 (44%) FH(–) patients presented carditis (p < .001). During follow up the severity of intestinal metaplasia increased by 0.4 ± 0.1 grades in FH(+) patients while remained unchanged in FH(–) patients (p < .001). Five 4% FH(+) and no FH(–) patients developed low-grade dysplasia (p = .02). Carditis regression was more frequent after H. pylori eradication.

Conclusions: 1. Intestinal metaplasia of the cardia, but not carditis is more frequent in GERD FH(+) patients. 2. Carditis regresses less frequently after H. pylori eradication in FH(+) GERD patients. 3. Intestinal metaplasia of the cardia worsens and dysplasia develops more rapidly in FH(+) GERD patients despite high-dose omeprazole treatment.

Abstract no.: P01.01
Pepsinogen I Levels and H. pylori in Siberian Populations
O. V. Reshetnikov,* S. A. Kurilovich,* S. A. Krotov† and V. A. Krotova†
*Institute of Internal Medicine, Novosibirsk, Russia; †Joint-Stock Company Vector-Best, Novosibirsk, Russia

Background: Low serologic pepsinogen I (PGI) value is a reliable surrogate marker of atrophic gastritis. The relationship between atrophic gastritis and Helicobacter pylori infection in Russia is still uncertain because of various climatic, socioeconomic, and ethnic differences.

Aim: To detect atrophic gastritis and H. pylori infection in four Siberian populations using serum biomarker tests.

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Conclusions: The prevalence of atrophic gastritis in Caucasians in Russia is higher than in European countries, in Asians being higher than in Japan. The association of atrophic gastritis and CagA positivity differs among populations and needs further investigation.

Abstract no.: P01.02
Sera were tested for PGI using ELISA (Gastropanel, Biohit). Atrophic gastritis was defined if PGI level was < 25 ng/mL. Anti-H. pylori IgG CagA antibodies were evaluated with domestic ELISA kits (Joint-Stock Company Vector-Best, Novosibirsk, Russia).

Results: The prevalence of atrophic gastritis was the lowest in urban Caucasians in Novosibirsk and significantly higher in Asian populations (p = .001). In contrast, the prevalence of H. pylori CagA infection was similar in three populations, being significantly higher in rural Asians (p = .03).

<table>
<thead>
<tr>
<th>Group</th>
<th>Atrophic gastritis</th>
<th>H. pylori infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>9.1%</td>
<td>72.7%</td>
</tr>
<tr>
<td>Group 2</td>
<td>12.3%</td>
<td>74.1%</td>
</tr>
<tr>
<td>Group 3</td>
<td>15.3%</td>
<td>72.2%</td>
</tr>
<tr>
<td>Group 4</td>
<td>26.7%</td>
<td>84.8%</td>
</tr>
</tbody>
</table>

Conclusion: The prevalence of atrophic gastritis in Caucasians in Russia is higher than in European countries, in Asians being higher than in Japan. The association of atrophic gastritis and CagA positivity differs among populations and needs further investigation.
**Abstract no.: P01.02**  
**Development of Serologic Method for Screening of *H. pylori* Infection in High-Risk Patients**

*Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran; Department of BCG, Pasteur Institute of Iran, Karaj, Iran*

**Introduction:** *Helicobacter pylori* is well known as causative agent of chronic gastritis, peptic ulcer, or gastric cancer. The diagnosis of infection based on endoscopy is an invasive and expensive method. Attempts are thus made in developing accurate noninvasive or less invasive detection methods which may replace endoscopy. The highly immunogenic protein, CagA, is a virulence marker of *H. pylori* with C-terminal polymorphisms due to different repeats of EPIYA-C segment. The aim of this study was to design a serologic method for diagnosing high-risk infected individuals.

**Methods:** The number of CagA EPIYA motifs was analyzed by specific PCR among Iranian *H. pylori* strains. N-terminal fragment of cagA from the most frequent subtype and four different fragments of C-terminal region with different EPIYA-C repeats were cloned in pTZ57R/T and then subcloned into the expression vector, pET32a. Expression of recombinant proteins was confirmed by Western blotting using anti-His tag antibody and *H. pylori* infected and noninfected sera.

**Results:** Ninety kDa recombinant protein related to N-terminal conserved region and four 87, 79, 76, 73 kDa r-proteins with three, two, one, and no EPIYA-C segment were expressed, respectively. These recombinant proteins were confirmed by Western blotting. Seroreactivity against these proteins was determined by a panel of *H. pylori*-infected patients.

**Conclusions:** Inclusion of CagA recombinant proteins with different EPIYA-C segments in serologic screening approaches may aid to detect patients infected with high-risk *H. pylori* strains for population screening purposes.

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**Abstract no.: P01.03**  
**H. pylori Immunoreactive Antigens and their Screening Applications**

Y. Talebkhan, F. Ebrahimzadeh, M. Esmaeili, A. Oghalaie, M. Sadeghi and M. Mohammadi  
*Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran*

**Introduction:** *Helicobacter pylori* is one of gastrointestinal pathogens which infects 50% of the adult population worldwide. Among outer membrane proteins of *H. pylori* Omp18 is a well-defined protein recognized by immune system. It is a peptidoglycan-associated lipoprotein precursor with low sequence similarity with close bacterial families. The aim of this study was to explore the immunologic criteria of r-Omp18 and other immunoreactive antigens of *H. pylori* for applications in screening approaches.

**Methods:** Recombinant plasmid was constructed and Omp18 was expressed in *E. coli* BL21 and tested in a home-made Western blotting assay. Recombinant urease A and B were also included in immunoblotting assay and obtained results were compared with available commercial kit. Data were analyzed using SPSS.

**Results:** Amplified 570 bp fragment encoding a 25 kDa Omp18 was cloned and induced by IPTG. By comparing blotting results with gold standard tests, sensitivity, specificity, accuracy, positive predictive value and negative predictive value of 94%, 90%, 93.3%, 97.4%, and 79.4% were determined for this r-protein, respectively. Statistically significant kappa values for the agreement between seropositivity towards the mentioned proteins and *H. pylori* ELISA serology were observed for 30 kDa and 116 kDa proteins of HelicoBlot2.1 as well as home-made Omp18 (kappa values 0.443, 0.321, and 0.72, respectively). Omp18 seroreactivity was associated with atrophic and intestinal metaplastic changes of gastric mucosa (*p* < .05).

**Conclusion:** Due to low heterogeneity of omp18 gene and protein sequences, it can be assumed as a conserved antigen. Comparing the observed results with gold standard tests demonstrated a potential application for including this protein in *H. pylori* serologic diagnostic tests.

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**Abstract no.: P01.04**  
**Erosive and Ulcerative Lesions of Gastrroduodenal Zone in Patients with Leukemias: Role of *H. pylori***

I. Skrypnyk and G. Maslova  
Ukrainian Medical Stomatological Academy, Poltava, Ukraine

Fifty-eight percent of cases of acute leukemia (AL) are accompanied by lesions of gastroduodenal zone. The purpose of this work is to evaluate influence of *Helicobacter pylori* association on pathogenesis of erosive and ulcerative lesions of gastroduodenal zone.

Seventy-three patients suffering from AL were observed with the concomitant diseases of the gastrointestinal tract (GT). Patients were distributed into two groups depending on the presence of *H. pylori*: group I (n = 44) – positive and group II (n = 29) – negative.

The study of mucous barrier of gastroduodenal zone was conducted by an uninvasive method, taking into account the gravity of the patient’s state.

The increase of concentration of *N*-acetylmuramic acid (NANA) was set in the blood serum in patients of group I – in 1.4 times, groups II – in 1.25 times in comparison with the norm and increase of excretion level of NANA in urine – in 1.5 and 1.3 time accordingly (*p* < .01).

Concentration of fucose, connected with the albumen, in blood serum went down in group I – in 2.5 times, to group II – in 2.1 times as compared to a norm, and fucose excretion with urine decreased in 2.3 and 1.7 times accordingly (*p* < .01).

**Conclusion:** In *H. pylori*-positive patients with LA more expressed decrease of mucous barrier resistance of gastroduodenal zone is marked, that is adequately evaluated by screening uninvasive method and can be the criterion of prognostication of chemotherapy complications in GT. *H. pylori* strengthens degradation of mucus protective albumens, perifocal inflammation, and reduce production of protective fucoproteins.
Abstract no.: P01.05
Accuracy of Ultrarapid Urease Test for the Diagnosis of *H. pylori* Infection


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**Background:** Rapid urease test (RUT) is a simple, cheap and relatively fast method to diagnose *Helicobacter pylori* infection.

<table>
<thead>
<tr>
<th>Test Type</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>Positive predictive value (95% CI)</th>
<th>Negative predictive value (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RUT</td>
<td>82.3% (76–89%)</td>
<td>88.4% (83–94%)</td>
<td>86.4% (81–92%)</td>
<td>84.7% (79–91%)</td>
</tr>
<tr>
<td>URUT (5 min)</td>
<td>57.8% (49–66%)</td>
<td>97.0% (94–100%)</td>
<td>94.9% (91–99%)</td>
<td>70.7% (63–78%)</td>
</tr>
<tr>
<td>URUT (30 min)</td>
<td>76.6% (69–84%)</td>
<td>95.6% (92–99%)</td>
<td>94.2% (90–98%)</td>
<td>81.3% (75–88%)</td>
</tr>
<tr>
<td>URUT (60 min)</td>
<td>84.8% (79–91%)</td>
<td>93.0% (89–97%)</td>
<td>91.8% (87–96%)</td>
<td>86.8% (81–93%)</td>
</tr>
<tr>
<td>RUT (no PPI)</td>
<td>87.8% (80–95%)</td>
<td>93.3% (88–99%)</td>
<td>94.7% (90–100%)</td>
<td>84.8% (77–93%)</td>
</tr>
<tr>
<td>URUT (no PPI)</td>
<td>90.9% (84–97%)</td>
<td>96.8% (93–100%)</td>
<td>97.6% (94–100%)</td>
<td>88.2% (81–96%)</td>
</tr>
</tbody>
</table>

Abstract no.: P01.06
Precision of Isotope-Selective Nondispersive Infrared Spectrometers (NDIRS) is not Satisfactory when Using Diabact UBT 50 mg 13C Urea Breath Test with Cut-Off 1.5 and Urease Incubation 10 Minutes

V. Skar, G. Mandic,† H. Hope* and C. R. Borge*

†Lovisenberg Diakonale Hospital, Oslo, Norway; †Oslo University Hospital, Oslo, Norway

**Introduction:** Nondispersive infrared spectrometers (NDIRS) compete with IRMS in 13C-based breath testing. New models of NDIRS with autosampler for 12 mL exeters make it possible to run “IRMS-kit” on this type of less costly and cumbersome instruments. It is unclear, however, if NDIRS precision is sufficiently high when using this new and lower sample volume. **Aim:** To compare IRMS (Abca 20–20, Europa Scientific, Crewe, UK) and NDIRS (Iris-lab, Wagner Analyzen Technic, Bremen, Germany) by running Diabact UBT 50 mg kit (Orexo AB, Uppsal, Sweden) samples on both instruments.

Therefore, it is the method of choice used in patients undergoing gastroscopy. Most kits require 24 hours to give results. The new ultrarapid urease test (URUT) kit by Biohit requires less than 1 hour.

**Aim:** To determine URUT’s diagnostic accuracy.

**Methods:** Prospective, blind, multicenter study including dyspeptic patients. Three antrum and one corpus biopsies were obtained during endoscopy for standard histologic analysis, RUT, and URUT. URUT result was checked after 1, 5, 30, and 60 minutes while RUT was checked out over 24 hours. Accuracy tests were performed considering histology as gold standard.

**Results:** One hundred and thirty-nine patients have been included so far, 70% female, mean age 49 years, 43% taking proton pump inhibitors (PPIs), and 49% *H. pylori* positive. RUT and URUT diagnosis were correct in 85.4% and 88.5% of the cases, respectively. Mean waiting time for positive RUT result was 6.5 hours. Subanaylsis based on the result at each temporal check point and on the intake of PPIs are shown in Table P01.05.01.

**Conclusion:** URUT is equivalent to (or even slightly better) traditional rapid urease tests in the diagnosis of *H. pylori* infection, and provides results in less than an hour.

Materials and Methods: Four hundred and ninety-seven consecutive samples from the same number of patients, 252 females, mean age 57 years, were analyzed from November 2008 to May 2009. IRMS results were considered gold standard and values < 1.5 delta over baseline (DOB) negative.

**Results:** Ninety-three of the 99 positive tests were positive on NDIRS, giving a sensitivity of 94%. Three hundred sixty-four of 398 were negative, with 91% specificity. In the majority of the false positive tests at least one tube was technically inferior with low CO2 content.

**Conclusion:** The high number of NDIRS false positive tests (34) when using 1.5 DOB cut-off is not acceptable. Reference tubes with software correction for drift would improve the IRIS results. Increase of the urease incubation time to 20 minutes would probably separate positives and negatives better, hopefully making an increase of Diabact cut-off to 3.5 DOB possible. The lower sensitivity of the NDIRS makes the instrument vulnerable when working with 12 mL samples instead of the customary bags with volume 40–300 mL.
Abstract no.: P01.07
Does Low Dosage of $^{13}$C-Urea Keep Good Accuracy for Diagnosis of $\text{H. pylori}$ Infection?


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Background: Recent study suggests that the dose of the substrate in $^{13}$C-urea breath test (UBT) can be substantially reduced without loss of accuracy (Gut 2006;55:455–62).

Aim: To assess the diagnostic accuracy of UBT containing 25 mg of $^{13}$C-urea comparing with the standard dosis of 75 mg of $^{13}$C-urea in the diagnosis of $\text{Helicobacter pylori}$ infection.

Patients and Methods: We invited 270 adult patients (96 males, 174 females, median age 41 years) to perform the standard UBT (75 mg $^{13}$C-urea) and to repeat the UBT using only 25 mg of $^{13}$C-urea within a 2-week interval. Participants who had used antibiotic in the last month and/or proton pump inhibitor in last week were excluded. The test was performed using an infrared isotope analyzer (IRIS, Wagner Analysen Technik, Germany). Patients were considered positive if DOB was > 4.0‰ at the gold standard test.

Results: One hundred and sixty-one (59.6%) patients were $\text{H. pylori}$ negative and 109 (40.4%) were positive by the gold standard test. By ROC analysis was established a 3.4‰ value as cut-off for 25 mg UBT [lowest values for false positive and false negative (%)] according to the $\text{H. pylori}$ prevalence [95% confidence interval (CI)] reported in the table. Diagnostic accuracy of 25 mg UBT: 92.9% (95% CI: 88.1–97.9); sensitivity 83.5% (95% CI: 75.4–89.3); and specificity 99.4% (95% CI: 96.6–99.9).

Conclusions: Low-dose UBT (25 mg $^{13}$C-urea) does not reach accuracy sufficient to be recommended in daily practice in regions with $\text{H. pylori}$ prevalence around 30%. False positive and false negative findings according to the 30.2% $\text{H. pylori}$ prevalence (95% CI: 23.9–37.3).

<table>
<thead>
<tr>
<th>95% CI $\text{H. pylori}$ prevalence</th>
<th>False positive (%)</th>
<th>False negative (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>23.9%</td>
<td>2.31</td>
<td>4.96</td>
</tr>
<tr>
<td>37.3%</td>
<td>1.24</td>
<td>9.00</td>
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Abstract no.: P01.08
Precision of isotope-Selective Nondispersive Infrared Spectrometers (NDIRS) and Isotope Ratio Mass Spectrometers (IRMS) When Using Autosampler and 12 mL Exetainers

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Background: Validation of precision, stability, and sensitivity of nondispersive infrared spectrometers (NDIRS) compared to isotope ratio mass spectometry (IRMS) has earlier been based on NDIRS instruments using sample volumes from 40–300 mL.

Aim: To test precision of NDIRS (Iris-lab, Wagner, Bremen, Germany) compared to IRMS (Abca 20-20, Europa Scientific, Crewe, UK), both instruments equipped with autosampler accepting 12 mL exetainers (Labco Ltd., High Wycombe, UK).

Method: Eight basal samples were produced by one of the authors (VS) by expiring directly into exetainers using a straw. Four and six basal samples, IRMS and NDIRS respectively, were always placed in front of the reproducibility series of eight basal samples. Baseline values were used in the calculations of standard deviation (SD) and range within the series. One hundred and thirty-five series were run on the NDIRS and 27 series on the IRMS from November 2008 to May 2009. Results are presented as percentiles (p). Student’s t-test was used when comparing groups.

Result: 90p (SD) for drift corrected IRMS, uncorrected IRMS, and NDIRS were 0.16, 0.40, and 0.75, respectively. The corresponding values for range were 0.49, 1.14, and 2.10. The Iris instruments recommended upper limit for reproducibility series of eight samples, SD > 0.5, was reached in only 50% of our series, mainly due to systematic drift.

Conclusion: IRMS showed significantly ($p < .0001$) better precision than NDIRS even when using uncorrected values. Iris does not offer drift correction. The results obtained with Iris are probably representative for IRMS in general and important to consider when using the autosampler version of these instruments.

Abstract no.: P01.09
Factors Influencing the Presence of Ammonia in Breath Air: Determining Sensitivity of the Test-System HELIC for $\text{H. pylori}$ In vivo Diagnostics

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Background: Noninvasive diagnostics of $\text{Helicobacter pylori}$ with test-system HELIC is based on kinetic registration of ammonia concentration in patient’s breath air after a portion of unlabeled urea C12 is taken. Using ammonia as a marker causes difficulties due to its low concentration and changeable presence, which depends on a number of factors.

Aim: To analyze factors that influence sensitivity of the test-system HELIC.

Study: Indicator tubes with different sensitivity were examined. A model for steam–gas mixture was produced in test stand (30.7 °C and humidity level > 80%) in order to evaluate sensitivity of the indicator tubes. Ammonia presence was determined if the indicator tube changed color. The influence of the way ammonia gets into the indicator tube was studied: the speed of the air flow and the sample volume. Also, the shifts for ammonia threshold levels were studied. Major meteologic characteristics were evaluated with mathematical statistics methods. Dispersion analysis was performed using Fisher and Kohren criteria.

Result: Decrease in humidity of the air sample blurs the border of the part that has changed color in the indicator tube, while the temperature increase shortens the air sample. The necessary sample volume is 0.67 L. Sensitivity range of the indicator tubes for

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Abstract no.: P01.10
Method of Breath Sampling for Noninvasive H. pylori Analysis

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Background: Urea breath test (UBT) with ammonia detection for Helicobacter pylori after taking normal isotope 14C-urea was analyzed.

Aim: To evaluate and improve the ammonia UBT method and breath sampling for different ages.

Materials and Methods: We used the results of breath tests made by Helic-device® for 2009 patients (3–84 years old). These results were received in 18 medical clinics from 2005 to 2009.

Changing of ammonia concentration in breath was analyzed by mathematical statistics and special PC program. The method is the result of the goal function optimization that maximizes the information content with minimization of the examination time.

Results: Ammonia concentration change in patient oral cavity after ingestion of 14C-urea has pronounced age dependence. The duration of ceaseless ammonia concentration increases for the first 9 minutes after 14C-urea ingestion depending on the age. The speed of ceaseless ammonia concentration increases and its increase value rapidly falls with increasing age for the age from 3 to 20 years old and stabilizes for the elder age. The basic ammonia concentrations for different age groups were the same. This is not only because of the age changes of anthropometric human characteristics (the gullet length, body weight, stomach surface area, etc.), but also because of physical changes in the condition of the digestive organs and according to previous diseases.

Conclusion: Breath sampling tactics were developed for different age groups. Methodology makes up rules for patient pretreatment, method, and identification duration of basic ammonia concentration, the method of 14C-urea ingestion, starting time, and period of breath sampling.

Abstract no.: P01.11
Immunity Marker of H. pylori in Adult Patients with Dyspepsia: Autoantibodies Against Parietal Cells (PCA)

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Aim: To evaluate the role of autoantibodies against parietal cells (PCA) in Helicobacter pylori infection in relation with histopathologic and serologic variables.

Methods: One hundred and twenty-four untreated dyspeptic patients (31 males, 93 females; mean age, 46.20 ± 12.70 years) who were admitted and referred to upper endoscopy at Dokuz Eylül University Hospital, Gastroenterology Polyclinic and Endoscopy Unit, were evaluated between April 2006 and February 2008. Two antrum and corpus biopsies and sera were taken from each patient. Rapid urease test (RUT) and histopathology were the gold standard methods. H. pylori-specific serum antibodies were detected by anti-H. pylori IgG and CagA IgG ELISA, anti-H. pylori IgG Western blot tests. Autoantibodies against PCA in monkey stomach as a substrate were determined by the indirect fluorescence test (EUROIMMUN Medizinische Labordiagnostika, Lübeck, Germany).

Results: Twenty-three patients had autoantibodies against parietal cells; 17 of them were H. pylori infection positive according to the gold standard methods. We observed no relationship between histopathology results (atrophy, intestinal metaplasia, and active chronic gastritis) and anti-H. pylori IgG, CagA IgG ELISA, and anti-H. pylori IgG Western blot tests.

Conclusion: We concluded that there is no correlation between anti-PCA and histopathology results, anti-H. pylori IgG, CagA IgG ELISA, anti-H. pylori IgG Western blot tests. Interestingly, many patients had autoantibodies against parietal cells. H. pylori might play an important role to induce the stimulation of parietal cells and autoimmune response at the gastric level.

Abstract no.: P01.12
Evaluation of Two Monoclonal-Based Antigen in Stool Enzyme Immunoassay for Diagnosis of H. pylori Infection in Spanish Children

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*Hospital Universitario La Princesa, Madrid, Spain; †Hospital Universitario Doce de Octubre, Madrid, Spain

In this study, we use two different Helicobacter pylori stool antigen tests as non-invasive diagnostic methods and we compared with diagnosis based on endoscopic biopsy-based methods (culture and urease test).

Fifty samples of biopsies obtained from pediatric patients with gastric symptoms were received at the Department of Microbiology (Hospital Universitario La Princesa, Madrid) from January 2006 to November 2008 and were cultured according to standard microbiologic procedures. Children also donated a sample of stool. Stool specimens from these patients were examined by rapid STRIP HpSA and one step simple H. pylori antigen cassette test for the detection of H. pylori antigens. Both of them are commercially available enzyme-linked immunosorbent assay-based technology. The sensitivity and specificity were calculated for non-invasive test used in this study.

For these 50 children, 40 (80%) were diagnosed as positive and 10 (20%) were diagnosed negative for H. pylori infection by the gold standard methods (culture and urease). Whereas 37 (74.5%) were positive and 13 (26%) were diagnosed negative by the rapid STRIP HpSA test. The sensitivity and specificity were 92.5% and 100%, respectively. By the simple H. pylori stool antigen cassette test, 39 patients (78%) were positive, and 11 (22%) were negative. The sensitivity and specificity were 97.5% and 100%, respectively.
Both stool antigen tests had high sensitivity and specificity for diagnosis of *H. pylori*. The noninvasive test could be used as a routine diagnostic tool in the microbiology laboratory for assessing clinical significance and eradication control of *H. pylori*.

**Abstract no.: P01.13**

**The Use of Histology as a Gold Standard for Diagnosis of *H. pylori* in Nigeria**


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**Aim:** Appropriate diagnostic method/s for *Helicobacter pylori* in Nigeria is still sought for. The aim of this study was to investigate the various methods available in Nigeria for the diagnosis of *H. pylori* and confirm with histology as the gold standard.

**Methods:** A total of 167 patients presenting with various gastroduodenal diseases were screened for the presence of *H. pylori* and compared with histology as the gold standard.

Three biopsies (after informed consent) each were taken from the patients (two from antrum and one from corpus). The biopsies were used for histology, CLO test, and direct gram stain. Stool samples were taken for *H. pylori* stool antigen test (Meridien). Blood samples were also taken for serology test using the kit from Human (Wiesbaden, Germany).

**Results:** Using the various tests, histology was positive in 32.7% of cases, HpSA 36.7%, CLO 26.4% of cases, and blood serology 41.7% of cases, while direct gram stain was positive in 18.9% of cases.

**Discussion and conclusion:** Due to difficulty in *H. pylori* culture as a result of increasing power outages, histology was now advocated as a gold standard. The histology results correlated with most positive cases of HpSA as well as serology. Serology is not reliable for *H. pylori* diagnosis. Urea breath test is the gold standard for noninvasive test, but few laboratories have the machine. The best method/s for therefore diagnosing *H. pylori* in Nigeria when histology cannot be affordable is HpSA and serology.

Dr. SI Smith is grateful to ICGEB CRP/NIG07-02 and NIMR for study sponsor.

**Abstract no.: P01.14**

**Production of Polyclonal Antibody against Alkyl Hydroperoxide Reductase (AhpC) of *H. pylori***

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Stool-antigen detection kits for diagnosis of *Helicobacter pylori* infection have been widely used because of their full noninvasive nature. Because *H. pylori* strains show a distinctive genetic diversity, it is important to find a protein that is a common antigen of various strains and shows a strong immunogenicity for the development of a stool-antigen detection kit. Alkyl hydperoxide reductase (AhpC) of *H. pylori* strongly reacts with the sera of patients with gastritis and peptic ulcer. Therefore, AhpC seems to be an excellent candidate as a target protein for this study. Accordingly, polyclonal antiserum against AhpC was produced in adult New Zealand white rabbits by using AhpC in the gel bands without adding Freund’s adjuvant.

In this study, a simple method was used for rapid production of polyclonal antibody against AhpC of *H. pylori*, which avoids both the long-term AhpC purification and the addition of Freund’s adjuvant. One-dimensional preparative gel electrophoresis allows a single and short purification step; the high-resolution capacity of this technique leads to a high level of purity of the protein and consequently to a very high specificity of the antibody. Moreover, this method avoids contamination by other non-specific proteins which often appear during the purification process by column chromatographic techniques, which may also decrease the purity of the immunogen.

The present method is simple, rapid and cost-effective, and it also makes it possible to produce antibody for stool-antigen enzyme immunoassay in a short time and at low cost.

**Abstract no.: P01.15**

**The Clinical Usefulness of the Noninvasive Rapid Urine Test to *H. pylori*: RAPIRUN®**


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**Background/Aim:** The aim of this study was to evaluate the diagnostic accuracy of RAPIRUN® test in clinical practice.

**Methods:** A set of *Helicobacter pylori* tests which were composed of endoscopic biopsy, 13C-urea breath test (13C-UBT), serum IgG-ELISA, and urine anti-*H. pylori* IgG test was conducted on
The prevalence of *H. pylori* was calculated using each test independently.

**Results:** The proportion of positive result of *H. pylori* test was 59.3%, 56.4%, 57.4%, and 50.5% with gastric mucosal biopsy, 13C-UBT, serum IgG-ELISA, and rapid urine RAPIRUN® test, respectively. With gastric mucosal biopsy, 13C-UBT, and serum IgG-ELISA as the gold standard, a patient was considered to be *H. pylori* positive when all three tests were positive, or *H. pylori* negative when all were negative. The sensitivity, specificity, positive and negative predictive value, and accuracy of the rapid urine RAPIRUN® test were 83.5%, 98.3%, 98.7%, 79.4%, and 89.3%, respectively.

**Conclusions:** Urine-based RAPIRUN® test for detection of anti-*H. pylori* antibody was an accurate test, especially in specificity. With the advantage of easiness, rapidity, and noninvasiveness of RAPIRUN® test, we expect that RAPIRUN® test would be useful in general practice for *H. pylori* screening.

**Abstract no.: P01.16**

**Evaluation of 13C-Urea Breath Test in Determining H. pylori Status After Treatment: About 197 Cases**

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**Aim:** To evaluate 13C-urea breath test (UBT) versus rapid urease test (RUT) history after treatment of *Helicobacter pylori* infection in Algerian adult patients.

**Methods:** In this prospective study, *H. pylori* testing was performed in 197 consecutive adult dyspeptic patients [mean age: 33.2 years; males: 45; nonulcer dyspepsia (NUD): 172; duodenal ulcer (DU): 25] not using proton pump inhibitor (PPI) or antibiotics during the last 4 weeks before testing. Each patient has had UBT, histology, and RUT (Pronto Dry). *H. pylori* infection was defined when RUT and histology were positive and was absent when the two tests were negative. All patients have been treated by different tritherapies. Eight to 12 weeks after the end of therapy, they have been re-evaluated by the three tests. *H. pylori* eradication was confirmed on the negativity of RUT and histology. The failure of the treatment was attested by the negativity of one or the two tests.

**Results:** After treatment, 61 patients had a positive histology and RUT. Among these patients, UBT was positive in 60 (98.3%) cases. On the other hand, 136 were negative for histology and RUT. One hundred and thirty-one (96.3%) among them were UBT negative. When compared to RUT and histology, sensitivity, specificity, positive predictive value, and negative predictive value of UBT were respectively: 98% [95% confidence interval (CI) = 77–99]; 97.3% (95% CI = 82–95.6); 93.7% (95% CI = 85–100); and 97.7% (95% CI = 91–100). UBT accuracy was 96.2%.

**Conclusions:** UBT is a highly sensitive and specific test to control the efficacy of the treatment of *H. pylori* infection in adult patients.

**Abstract no.: P01.17**

**New Diagnostic Method for the Detection of Clarithromycin Resistance in H. pylori Strains Using Peptide Nucleic Acid Probes**

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**Helicobacter pylori** is a Gram-negative rod that colonizes the human stomach and is associated with the gastrointestinal disorders such as chronic gastritis, peptic ulcer disease, and gastric carcinoma. The over usage of the standard treatments (triple therapy) led to an increased antibiotic resistance. So far, routine antibiotic-resistance characterization of pathogens in clinical and environmental samples relies on the fastidious and time-consuming plating methods. This work intends to develop and apply fluorescence in situ hybridization (FISH) with peptide nucleic acid probes (PNA) to rapidly and specifically detect and characterize antibiotic resistance of *H. pylori*.

Three mutations in the *H. pylori* 23S rRNA are strongly associated with clarithromycin resistance. In these mutations, an adenine is replaced by a guanine at positions 2142 and 2143, or a cytosine at position 2142. Hence, we developed a set of PNA probes for the identification of target sequences for the different clarithromycin resistance polymorphisms. PNA molecules are DNA synthetic mimics with a noncharged backbone, due to their chemical configuration. As such, they present a lack of electrostatic repulsion, resulting in improved thermal stability compared with DNA/DNA duplexes.

After probe design, an optimization of the hybridization conditions, such as temperature, pH, ionic strength, and formamide concentrations, was performed. To ensure specificity and sensitivity, probes have been tested against resistant and susceptible strains of *H. pylori*.

This novel PNA FISH method will facilitate a more prompt diagnosis of *H. pylori* clarithromycin resistance directly in clinical samples such as gastric biopsy specimens, thus allowing a more rational patient treatment.

**Abstract no.: P01.18**

**Delayed Positivity of Rapid Urease Test: Is There Any Clinical Significance?**

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**Background-Aim:** Rapid urease tests (RUT) are widely used because of their simplicity and reliability. It is not uncommon though, the tests to render late (≥ 72 hours) positivity, although...
initially negative (≤ 24 hours). It is not clear if it is due to a false positive result or either slow production of urease from other urease-positive bacteria or if it is related to less densely populated gastric mucosa with *Helicobacter pylori*.

**Patients and Methods:** One hundred and thirty-six patients (44 male, 92 female), endoscoped for dyspepsia, heartburn, or anaemia, were studied. Exclusion criteria included current or recent (≤ 4 weeks) use of proton pump inhibitors, H₂ antagonists and/or antibiotics and the presence of active upper gastrointestinal bleeding. A double gel-based RUT (Hut-Test AstraZeneca GmbH) and histology from both the antrum and the corpus (two specimens) were performed. RUT tests were assessed at 24 hours (early) and 72 hours (delayed) and were compared with histology.

**Results:** Eighty-four of 136 (62%) patients had either positive (30 of 84) or negative (54 of 84) results to both (early and delayed) RUT readings. In 80 of 84 (95%) histology was in concordance with RUT, while in the rest four out of 84 (5%) there was discrepancy between RUT and histology. Fifty-two of 136 (38%) presented delayed positivity of RUT test, with the early reading being negative. In 18 of 52 (34%) [18/136 (13%) of total] histology results were in concordance with the delayed positive reading, confirming the presence of *H. pylori*, while in the rest 34 of 52 (66%) histology was negative for *H. pylori*.

**Conclusions:** It seems that RUT turns to render a true late (≥ 72 hours) positivity in about 15% of patients tested for *H. pylori*.

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**Abstract no.:** P01.19

**H. pylori Detection and Susceptibility Testing: Histopathology Through Culture to FISH**

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**Aim:** To evaluate a bouquet of diagnostic tests to detect *Helicobacter pylori* infection and to determine the clarithromycin susceptibility by culture and colony FISH method.

**Methods:** Eight adult patients with dyspepsia who had failed treatment underwent EGD at the Dokuz Eylül University Hospital. Culture and histopathology were the gold standard methods. Columbia blood agar (Oxoid) (10% human serum, antibiotic supplements, amphotericin B, and 0.3% activated charcoal) and also modified Helicobacter Agar (BD) were used. Antimicrobial susceptibility testing was performed on Mueller–Hinton Agar with 10% human serum by disc diffusion method (10 µg amoxicillin, 15 µg clarithromycin, 80 µg metronidazole, 5 µg ciprofloxacin, and 5 µg levofloxacin). FISH (BactFISH *H. pylori* Combi Kit) method was performed on both isolated *H. pylori* colonies and formalin-fixed paraffin-embedded tissue sections. All tests were performed on both antrum and corpus biopsies.

**Results:** Six patients were *H. pylori* positive by culture, histopathology, and also FISH from colonies and paraffin sections. Three patients had clarithromycin-susceptible strains by disc diffusion method, colony, and paraffin section FISH but they had resistant strains to metranidazole, amoxicillin, ciprofloxacin, levofloxacin by disc diffusion. Two patients had clarithromycin-susceptible strains by colony FISH but clarithromycin-resistance strains by paraffin section FISH and had resistant strains to clarithromycin and metranidazole by disc diffusion. One patient had clarithromycin-susceptible and -resistant strains by colony and paraffin section FISH and disc diffusion. Two other patients had positive histopathology but negative culture result; one had clarithromycin-susceptible; the other had clarithromycin-resistant strains by paraffin section FISH.

**Conclusion:** Both the paraffin section FISH and the colony FISH could be used instead of conventional antimicrobial susceptibility testing for clarithromycin when a quick decision is necessary for patients with treatment failure.

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**Abstract no.:** P01.20

**Estimating Working Life for HELPYL Rapid Urease Test**

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**Background:** HELPYL is a ready-to-use test for detection of *Helicobacter pylori* urease activity in biopsy specimen obtained by gastroscopy. Due to its dry surface the test allows to detect urease in positive samples without incubation. It takes 3 minutes to change the color of the indicator from yellow to blue. The same biopsy specimen can be examined with other methods such as culture, histology, etc. directly after the 3-minute testing. Applied in Russia for over 10 years, HELPYL proved to be highly sensitive (93%) and specific (95%).

**Aim:** To confirm sensitivity and specificity of rapid urease test after 6-month working life period.

**Methods:** We have compared sensitivity of tests in laboratory using model urease solutions (10 U/mL, 20 U/mL, 100 U/mL) for HELPYL stored 8–16 months. Also we compared sensitivity and specificity of “old” HELPYL (after the expiry date, 6 months) with the “fresh” HELPYL (before the expiry date) at real biopsy samples in hospital. Twenty-one HELPYL were stored over 12 months and were examined then. *H. pylori* urease activity in biopsy specimen was detected using “old” HELPYL and the “fresh” HELPYL at first and then histology was performed.

**Results:** Laboratory experiments proved same sensitivity of “old” HELPYL. Later in 20 hospital testings all the three methods gave the same results. Only one “old” HELPYL showed discrepancy with the “fresh” HELPYL and histology. Thus, discrepancy rate was 5%.

**Conclusion:** “Old” HELPYL has the same sensitivity and specificity and can be used to detect *H. pylori* urease activity in biopsy specimen for 12 months at least.
Abstract no.: P01.21
The Importance of the Positivity Duration for Rapid Urease Test in the Diagnosis of H. pylori Infection at Endoscopy Unit

E. Türkoğlu,† S. Kesici,* A. Meşen,† E. Yener,† E. Demiray Gürbüz,† M. Güvenir,‡ N. Bekmen,† S. Namli,‡ Ö. Sağol,‡ H. Ellidokuz,‡ M. Soytürk,‡ I. Şimşek‡ and Ö. Yilmaz†
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Abstract no.: P02.01
Primary Antibiotic Resistance of H. pylori Strains Isolated from Portuguese Children: A Prospective Multicenter Study

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Abstract no.: P02.02
Usefulness and Influence of Age of a Novel Rapid HpSTAR™ Stool Antigen for the Diagnosis of H. pylori Infection in Children

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P02 Pediatric Issues
Abstract no.: P01.21
The Importance of the Positivity Duration for Rapid Urease Test in the Diagnosis of H. pylori Infection at Endoscopy Unit

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Aim: To investigate the positivity duration of in-house rapid urease test (RUT) and to evaluate diagnostic results of endoscopy for Helicobacter pylori infection by the "H. pylori Student Special Study Module (HPSSM) Group" of the Third Year Medical School Students.

Methods: Sixty patients (32 males, 28 females; mean age, 53.26 ± 14.48 years) who were admitted with different clinical complaints and referred to upper endoscopy at Dokuz Eylül University Hospital, Gastroenterology Polyclinic and Endoscopy Unit, were randomly chosen and evaluated between April 20 and May 6, 2009. All patients had given informed consent. The gastric biopsies were taken from the antrum and corpus and were placed into RUT tubes, the reaction of urease activity immediately started and followed up for 24 hours. RUT was performed to all patients but histopathologic examination was required when necessary for the diagnosis of H. pylori infection. All data obtained were evaluated by SPSS version 11.0.

Results: The positivity of H. pylori infection by RUT was 35%. The median of RUT positivity duration was 10 minutes (1 < t < 315). Ten patients (47.6%) were positive in 10 minutes. Twelve (57.1%) of 21 H. pylori positive patients were negative on endoscopy reports but their results became positive in 24 hours. According to the endoscopy results, frequency of different diseases in the esophagus, stomach, and duodenum in these 60 patients was 73.3%, 91.7%, and 21.7%, respectively. Statistical differences were found between diseases in duodenum and RUT (Fisher’s exact test = 0.007). Seventeen (28.3%) patients were smoking, and no statistical difference was found according to the RUT positivity (p = .568). The positivity of H. pylori infection by histopathology was 55% in 20 patients. There was a correlation between histopathology and RUT in 16 patients.

Conclusion: The evaluation of RUT positivity should be continued at least 3–4 hours before giving RUT results in endoscopy reports. Moreover, RUT positivity was associated with duodenal disease according to the results of our study group.

P02 Pediatric Issues

Abstract no.: P02.01
Primary Antibiotic Resistance of H. pylori Strains Isolated from Portuguese Children: A Prospective Multicenter Study

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Aim: To prospectively assess the evolution pattern of resistance to antibiotics in Helicobacter pylori strains isolated from Portuguese children over the last 9 years.

Materials and Methods: From 2000 to 2008, 950 H. pylori strains were isolated from Portuguese children attending the three Pediatric Gastroenterology Units in the Lisbon area for upper gastrointestinal symptoms (50.9% males; age: 15.9% < 6 years, 46.5% 6–11 years, and 37.7% > 11 years). Antibiotic susceptibility testing to clarithromycin, metronidazole, tetracycline, ciprofloxacin, and amoxicillin, was performed by E-test and disk diffusion. For all cases, testing was carried out before the first treatment.

Results: H. pylori primary resistance rate was: 34.1% to clarithromycin, 13.4% to metronidazole, and 4.1% to ciprofloxacin. Simultaneous resistance to two of these antibiotics occurred in 6.6% of the isolates. Resistance to tetracycline and amoxicillin was not observed. H. pylori antibiotic resistance rate was not associated to gender or to children age. Considering 3-year periods, 2000–02 (n = 191), 2003–05 (n = 320), and 2006–08 (n = 439), no significant temporal trend was noticed for clarithromycin and metronidazole resistance, while resistance rate to ciprofloxacin has significantly increased over time (p = .099). The same temporal trend was observed for the double-resistant strains (p = .038).

Conclusion: Antibiotic resistance persists high among H. pylori strains from Portuguese children. These results are particularly relevant concerning clarithromycin resistance, reflecting the recognized overuse of macrolides in children in Southern European countries. Additionally, the increasing detection of ciprofloxacin-resistant and double-resistant strains should deserve surveillance, as it may compromise the efficacy of second-line H. pylori therapies at this age group.

Abstract no.: P02.02
Usefulness and Influence of Age of a Novel Rapid HpStART™ Stool Antigen for the Diagnosis of H. pylori Infection in Children

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Aim: To assess the usefulness and the age influence in children of a novel rapid monoclonal enzyme immunoassay stool antigen
Abstract no.: P02.03
Prevalence of H. pylori-Associated Corpus Predominant Gastritis in Children

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Introduction: Helicobacter pylori-associated gastritis is a risk factor for gastric cancer, especially corpus-predominant gastritis. Infection during childhood leads to prolonged exposition to this potentially cancerogenic agent.

Methods: From February 2006 to August 2008, 265 consecutive patients (St.-Anna-Kinderspital) underwent esophagogastroduodenoscopy (EGD); 28 were excluded from analysis (24 follow-up investigations, four incomplete data). In the analyzed group of 237 patients (134 female; median age 10.7; range: 0.4–25.0a) indication for EGD was: upper abdominal pain (n = 151/64%), celiac disease (n = 49/21%), suspected GERD (n = 17/7%), hematemesis (n = 6/3%), inflammatory bowel disease (n = 6/3%), and other indications (n = 51/2%). H. pylori infection was defined to be present when histology or culture was positive. Grade of inflammation was evaluated by the updated Sydney Scoring System (uSSS). Gastritis was defined to be antrum predominant when inflammation was higher by at least one/two points according to uSSS in the antrum, and corpus predominant vice versa.

Results: Eighty-eight (37.1%) were H. pylori positive, 149 patients had no detectable H. pylori infection. When classification of antrum versus corpus predominance was performed by two points according to the Sydney Scoring System, 59 patients (24.9%) had pangastritis, 27 (11.4%) antrum-predominant gastritis, and 1 (0.4%) corpus-predominant gastritis. When classification of antrum versus corpus predominance was performed by one point, 42 patients (17.7%) had pangastritis, 43 (18.1%) antrum-predominant gastritis, and 3 (1.3%) corpus-predominant inflammation.

Abstract no.: P02.04
Follow up of H. pylori Eradication Rate in Symptomatic Children: An 8 Years Experience in a Single Center

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Introduction: The eradication rate of Helicobacter pylori infection with standard triple therapies tends to decrease.

Aim: To evaluate the efficacy of four therapies used in the eradication of H. pylori in children.

Methods: We conducted a prospective randomized study on 688 consecutive symptomatic children (age range 6 months–18 years; 416 girls) with uninvestigated dyspepsia, referred for gastroscopy during the past 8 years (2001–2008). H. pylori infection was assessed before and 4 weeks after treatment by urease test and histopathology. Four hundred and twelve (59.88%) of the 688 children were H. pylori infected.

They were randomized in four groups to receive one of the standard three first-line triple therapies for 7–14 days consisting of omeprazole (O) plus two of the following: clarithromycin (C), amoxicillin (A), and metronidazole (M), or a 10-day sequential regimen with esomeprazole and amoxicillin for the first 5 days, followed by omeprazole, clarithromycin, and metronidazole. In patients failing to be cured by a first treatment, a second alternative was applied.

Results: Overall, H. pylori infection was eradicated in 325 children (78.88%) after the first treatment, with no significant statistical differences depending on the type of the first-line triple therapy used, varying between 75% for OCM to 83% for OAC. The eradication rate with the sequential regimen was statistically bigger than the one obtained with the standard treatment (86.66% vs 79%).

Conclusions: Our data suggest that the sequential regimen achieved a higher eradication rate of H. pylori infection in children, compared with the standard triple therapy used.

for Helicobacter pylori detection (“Rapid HpStAR™”, Thermo Fischer Scientific, Oxoid®, Cambridgeshire, UK).

Patients and Methods: Over 9 months, 108 unselected children (mean age 7.6 ± 4.8 years, 40 girls) were enrolled. Infection was proven by upper gastrointestinal endoscopy in the course of diagnostic evaluation of clinical gastritis, recurrent abdominal pain, nausea, and vomiting. Four (two antral and two corpus) biopsy specimens were taken and analyzed for histology (updated Sydney classification), rapid urease test, and culture. The H. pylori status (reference method) was defined as positive when culture was positive or two of the three tests were positive. Patients were also asked to provide before endoscopy a stool sample up to 24 hours, which was immediately stored at −80 °C until analysis.

Results: The “Rapid HpStAR™” was evaluated in 16 infected and 92 noninfected children. The overall sensitivity, specificity, and positive and negative predictive values were 87.5%, 97.8%, and 87.5% and 97.8%, respectively, with a test accuracy of 96.2%. The highest performance was observed in children less than 5 years with a sensitivity level of 100%, contrasting with a moderate level of 84.6%, in those above 5 years. A good negative predictive value was observed in all age groups and particularly in younger children achieving 100%.

Conclusion: The “Rapid HpStAR™” is a highly concordant, reliable and specific test for the detection of H. pylori infection in children.
Abstract no.: P02.05
Comparison of Esomeprazole and Lansoprazole in Eradicating H. pylori Among Children

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Objective: First-line eradication therapy for Helicobacter pylori infection in children is based on a proton pump inhibitor combined with two antibiotics. The success rates, depending on many factors, vary between 73–92%.

Aim: The aim of the study was to compare the effect of esomeprazole and lansoprazole among children infected with H. pylori.

Methods: Symptomatic children with H. pylori infection detected by 14C-urea breath test underwent upper gastrointestinal endoscopy. H. pylori gastritis was confirmed histopathologically by modified Sydney classification, then patients received 10-day treatment with amoxicillin, clarithromycin, and either lansoprazole (group 1) or esomeprazole (group 2) randomly. Succes of eradication was investigated by 14C-urea breath test 8 weeks after treatment.

Results: Ninety-eight children underwent upper gastrointestinal endoscopy and were randomly treated, 25 patients were lost during follow up, eventually 73 children aged between 6–18 years were included to the study. H. pylori gastritis was confirmed in all children histopathologically. Group 1 consisted of 37 and group 2 of 36 children. Treatment was successful in 25 of 37 (67.56%) and 32 of 36 (88.89%) in group 1 and group 2, respectively (p < .05).

Conclusion: H. pylori gastritis is commonly seen in children of a developing country. Esomeprazole seems to be more effective than lansoprazole.

Abstract no.: P02.06
Evaluation of a Monoclonal Stool Antigen Test for the Diagnosis of H. pylori Infection in Preschool Algerian Children

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Background: As noninvasive tests for Helicobacter pylori infection, stool antigen test have been widely used in children. In North Africa, however, data are lacking.

Aim: To evaluate the performance of HpSTAR (DakoCytomation GmbH, Hamburg, Germany), a stool antigen test based on monoclonal antibodies for the diagnosis of H. pylori infection in symptomatic children.

Methods: From 2006 to 2008, 78 symptomatic children (mean age 2.9 years; 7 months to 5 years) underwent upper endoscopy with gastric biopsies for recurrent abdominal pain, vomiting, chronic diarrhea, delay of growth, and anemia. None was previously treated for H. pylori infection and had received antibiotics, antacids, and proton pump inhibitors in the preceding 4 weeks. All have had the four following tests: histologic examination, rapid urease test (Pronto Dry, Medical Instrument Corp, France), culture, and HpSTAR. Diagnosis of infection was assessed if culture

and/or both histology and rapid urease test were positive, and absence of infection if all these tests were negative.

Results: HpSTAR was a conclusive test among all the children and yielded a positive result in 28 of the 28 infected children (sensitivity 100%) and in two of the 50 noninfected children (specificity 96%). The predictive values for a positive and a negative result were 93.3% and 100%, respectively.

Conclusions: HpSTAR is highly accurate for the pretreatment diagnosis of H. pylori in preschool children.

Abstract no.: P02.07
H. pylori Prevalence in Lithuanian Children with Functional Dyspepsia, Peptic Ulcer, and Erosive GERD

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Helicobacter pylori is implicated in the development of peptic ulcer disease, but its relation with gastroesophageal reflux disease (GERD) is still controversial especially in children.

Aim: To determine the prevalence of H. pylori infection among all consecutively children with chronic dyspepsia and establish infection rate in children with functional dyspepsia (FD), peptic ulcer (PU), and erosive GERD.

Methods: Endoscopy was performed on all symptomatic children (age 6–18 years) with chronic dyspepsia, and according to macroscopic changes the children were divided into three groups: FD, PU, and erosive GERD. During endoscopy four biopsy specimens were taken for histology, culture, and urease testing. Additionally fecal samples and serum for detection of H. pylori were obtained. Patients were considered to have H. pylori infection when culture or at least other two of recited tests were positive.

Results: We investigated 135 children with chronic dyspepsia, according to endoscopic findings – 75 consecutive children with FD, 31 consecutive with PU, and 29 consecutive with erosive GERD. In concordance with our chosen H. pylori diagnostic standard – 55.6% of dyspeptic children were H. pylori positive; 56.0% in FD, 77.4% in PU, and only 31.0% in erosive GERD group (p = .001).

Conclusion: We have found that H. pylori prevalence in Lithuanian schoolchildren with chronic dyspepsia decreased in the last decade; however, this remains still high. Children with erosive GERD were statistically less likely to be H. pylori positive than children with functional dyspepsia.
Abstract no.: P02.08
Antimicrobial Resistance of *H. pylori* in Children in Central Poland

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Introduction and Objectives: *Helicobacter pylori* resistance to antimicrobial agents is an important factor compromising the efficacy of eradication therapy. Therefore this study, which is a part of the Third European Multicentre Study on Antibiologic Susceptibility of *H. pylori*, aimed at analyzing the current primary resistance of *H. pylori* isolated from children in Central Poland.

Materials and Methods: *H. pylori* strains were isolated from gastric biopsy specimens obtained from children who underwent endoscopy between April 2008 and April 2009. Susceptibility testing to clarithromycin (CL), metronidazole (MTZ), amoxicillin (AMX), tetracycline (TC), levofloxacin (LV), and rifabutin (RB) was performed using Etest.

Results: A total of 56 *H. pylori* strains were isolated from children (25 females, 31 males, age range 3.5–18 years, median 13 years), who had never received eradication therapy. All isolates were susceptible to AMX, TC, LV, and RB. However, as much as 42.9% (24 of 56) of isolates were resistant to CL, and 21.4% (12 of 56) showed resistance to MTZ. CL and MTZ resistance showed significant changes compared to 2001–2004, when it reached 28% (*p* = .045) and 40% (*p* = .01), respectively.

Conclusions: Diverse trends of CL and MTZ resistance in *H. pylori* isolates from children have been observed in Poland in recent years. Increasing CL resistance could be attributed to the extensive use of macrolides in respiratory tract infections. However, the reason for decreasing MTZ resistance remains unknown. Alarmingly high CL resistance precludes the use of this agent in empirical eradication therapy in pediatric patients in Poland.

Abstract no.: P02.09
Rifaximin in Initial Treatment of *H. pylori*: A Pilot Pediatric Study

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Background: The eradication rates using 7-day standard triple therapy in children have fallen and are now typically 80% or less. In compliance with Maastricht III recommendations, quadruple therapy can be advisable treatment regimen for first-line therapy in certain regions of the world. Application of rifaximin for treating *Helicobacter* infection is a debatable theme until now.

Aim: To provide a pilot study of empiric rifaximin, rabeprazole, bismuth substrate, and amoxicillin quadruple therapy for *H. pylori* gastritis in childhood.

Materials and methods: Forty-six Russian pediatric outpatients (29 females, mean age 14.5 ± 1.4 years) with *H. pylori*-associated chronic gastritis who underwent endoscopy for dyspeptic symptoms received the combination of rifaximin (800 mg/day) for 10 days, bismuth subcitrate (8 mg/kg/day, four times a day) for 14 days, rabeprazole (1 mg/kg/day two times a day) for 14 days, and amoxicillin (50 mg/kg/day, two times a day) for 14 days. *H. pylori* status was determined before the treatment by modified Giemsa staining/urease test and after the treatment (in 4–6 weeks) by ammonia breath test.

Results: *H. pylori* was eradicated in 41 children (89.1%); 95% confidence interval: 79.9–98.2 intention-to-treat and per-protocol tests). There were no serious adverse reactions and no withdrawals due to any side-effects.

Conclusion: The combination of rifaximin, rabeprazole, bismuth substrate, and amoxicillin was an effective and tolerable regimen for initial *H. pylori* eradication in children in Russia.

Abstract no.: P02.10
Gastroesophageal Reflux Symptoms, *H. pylori*, and Associated Factors in Adolescents

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Objectives: Gastroesophageal reflux (GER) is widespread among the general population affecting 10–20% of adults in the Western world. However, there is a notable lack of epidemiologic data describing prevalence of GER in children. The aims of the present study were to assess the prevalence of GER symptoms in adolescents and to evaluate factors associated with GER including markers of *H. pylori* infection.

Methods: School students in grades 9–11 in four randomly selected secondary schools in Novosibirsk, Western Siberia, participated (449 adolescents, 189 boys, 260 girls aged 14–17). They completed the Bowl Disease Questionnaire and lifestyle questionnaire. Serum antibodies against *H. pylori* and CagA protein were detected using Pyloriset-New EIA-G (Orion Diagnostica, Espoo, Finland) and ELISA kits (Vector-Best Joint-Stock Company, Novosibirsk, Russia), respectively.

Results: GER symptoms on a monthly basis were reported by 22% of students, weekly GER was reported by 9% of adolescents with the same frequency in both genders. Both *H. pylori* and CagA positivity were associated with GER. Among *H. pylori*-negative subjects, GER was found in 16.8% compared to 25.2% in *H. pylori* positives (odds ratio (OR); 95% confidence interval (CI) = 1.7; 1.0–2.8). CagA-negative students experienced GER in 16.5% vs 30.3% of CagA-positives (OR; 95% CI = 2.2; 1.3–3.7). Additionally, GER was related to family history of dyspepsia or GER, mother’s lower educational attainment, overweight, unhealthy eating patterns, alcohol consumption, and smoking.

Conclusions: GER symptoms are common among adolescents. Some precipitated factors found in this study (namely *H. pylori* and CagA positivity) are modifiable and may be corrected in adolescent population.
P03 Epidemiology and Transmission

Abstract no.: P03.01
Evolution of H. pylori Infection Rate in Belgium

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Background: Prevalence of Helicobacter pylori infection is declining in the industrialised countries. In this retrospective observational study we analyzed the epidemiology of H. pylori infection in Belgium during the last 20 years.

Study: Data of H. pylori infection according to the culture results from patients attending several adult and pediatric endoscopy units were analyzed.

The yearly prevalence was calculated. For the purpose of comparison, patient’s age, gender, and ethnic background (particularly Northern and Western Europe and North Africa) were considered.

Results: From January 1988 to December 2007, a total of 52,566 gastric biopsies were cultured in the Microbiology Department of Brugmann University Hospital serving several centers.

Specimens were taken in the course of 32,037 endoscopies performed in 22,612 patients aged 1 to 99 years. The annual proportion of infected patients decreased gradually from 43.4% in 1988 to 29% in 2007. Significant differences were observed between ethnic groups. The prevalence observed among Northern and Western European patients decreased from 36.2% in 1988 to 15.2% in 2007, compared to a decrease from 71.7% to 40% in North Africans. Surprisingly, this trend of decline in the prevalence of H. pylori infection was not observed in North African children under the age of 9 years. Infection rate was lower in adult females compared to males.

Conclusion: This study highlights the variability of the prevalence of H. pylori infection among persons living in the same geographic area and probably reflects not only ethnic but also sociocultural and standard of living differences.

Abstract no.: P03.02
H. pylori Infection in Children: Population-Based Age-Specific Prevalence and Risk Factors in a Developing Country

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The prevalence, age of disease acquisition, and risk factors for Helicobacter pylori infection were determined in a cross-sectional study among children.

Methods: H. pylori infection was assessed with ELISA in children aged 1 to 15 years in a community-screening program in Karachi, Pakistan. Parents responded to a questionnaire on number of individuals in house, rooms, water source, type of latrines, and housing. Parents’ socioeconomic status (SES) was assessed by Hollingstead Index (HI) based on occupation, level of education, and income.

Results: A total of 1976 serum samples were tested. H. pylori seropositivity in 1 to 5 years was 194 (36.6%), 6 to 10 years 316 (47.2%) [odds ratio (OR) 1.95 95% confidence interval (CI) 1.2–1.95] and 11 to 15 years 414 (53.5%) (OR 2.0 95% CI: 1.6–2.5). It increased with crowding index of 2–4 to 45.9% (OR 1.23 95% CI 0.92–1.63) and to 51.2% with crowding index > 4 (OR 1.52 95% CI 1.12–2.06) compared to 40.8% with low crowding index. In middle SES, seropositivity was 331 (50.5%) (OR 1.7 95% CI 1.3–2.4) while in lower SES 500 (47%) (OR 1.5 95% CI 1.1–2.0). Multivariate analysis showed H. pylori seropositivity in 6 to 10 and 11 to 15 years was high (OR 1.5 95% CI 1.2–1.9 and OR 1.9 95% CI 1.56–2.47, respectively) and in lower to middle SES (OR 1.6 95% CI 1.2–2.1 and OR 1.5 95% CI 1.10–2.0, respectively) also in children with uneducated fathers (OR 1.5 95% CI 1.27–1.97).

Conclusion: H. pylori seropositivity is significant. It increases with age, low to middle socioeconomic, and fathers’ educational status.

Abstract no.: P03.03
Helicobacter pylori Infection and Risk of Biliary Tract Cancer Death in a Nested Case–Control Study in Japan

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Aim: To investigate the association between Helicobacter pylori infection and risk of biliary tract cancer death, a nested case–control study was conducted.

Subjects and Methods: Our nested case–control study was conducted within the JACC study, a large cohort study that included 127,477 participants who were 40–89 years of age at baseline (1988 to 1990) throughout Japan. Subjects were 88 cases who subsequently died from biliary tract cancer (ICD10: C23 and C24) during an 13-year follow up and 263 controls who were randomly selected from all noncases. The controls were matched to cases by area, gender, and age. Serum samples were collected at baseline. We measured H. pylori IgG antibody using HM-CAP and E-plate with antigen from Japanese by ELIZA, where cut-off value was 2.7 EV and 10 U/mL, respectively. The odds ratios (ORs) for biliary tract cancer death were calculated using logistic regression model with adjustment for gender, age, smoking habits, and drinking habits.

Results: H. pylori seroprevalence among the case and control subjects was 85.2% and 80.2%, respectively. The ORs for biliary tract cancer death was 1.31 (95% confidence interval: 0.73–2.36) in the H. pylori-positive subjects after adjustment for gender and
Abstract no.: P03.04
Profile of HIV–H. pylori Coinfected Patients in the Highly Active Antiretroviral Therapy Era

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Aim: Highly active antiretroviral therapy (HAART) is the current standard of care for HIV-infected patients. Some of these are infected with Helicobacter pylori. Few prospective trials have compared patient characteristics according to H. pylori status. This study was designed to correlate demographics and upper gastrointestinal endoscopic (UGE) findings with H. pylori status.

Methods: We prospectively included every HIV-infected patient under HAART who underwent UGE for the first time from January 2004 to December 2008. Data were collected on: demographics (age, gender, body mass index (BMI), tobacco habit, alcohol intake, HIV risk behaviors); comorbidities (viral hepatitis B or C, any organ dysfunction, and opportunistic diseases); medication including antibiotics, anti-H2 receptor or proton pump inhibitor and NSAID; CD4 cell count, viral load; and symptoms, endoscopic and histologic (H. pylori determined by Giemsa staining) diagnosis. Two groups of patients were compared according to H. pylori status (presence versus absence).

Results: Two hundred and twenty-seven patients were under HAART regimen; among which 141 were tested for H. pylori status. Those with H. pylori infection had a significantly higher BMI (p = .02) and, CD4 cell count (p = .00), more duodenal ulcers (p = .01), significantly lower rates of comorbidities (p = .03), viral load (p = .00), and use of antibiotics (p = .00). There was no statistically significant difference in all others demographic, medication, and endoscopic diagnosis between the two groups.

Conclusion: In the HAART era, HIV–H. pylori coinfection is associated with duodenal ulcer, and higher CD4 counts, higher BMI, and fewer comorbidities or use of antibiotics.

Abstract no.: P03.05
Factors Influencing the Efficacy of First-Line Treatment of H. pylori Infection in Algerian Patients

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Aim: To determine the factors influencing the efficacy of Helicobacter pylori infection treatment in Algerian adult patients.

Methods: In this prospective study, 272 consecutive adult H. pylori-positive dyspeptic patients (mean age: 33.07 years; males: 85%; nonulcer dyspepsia: 208; DU: 64) have been treated by four different first-line regimens: OAM, OAC, and RbmcCT for 7 days and OAM with a high dose of metronidazole (1.5 g) for 10 days. All patients were controlled 8–12 weeks after treatment. The eradication of H. pylori infection was attested by the negativity of four tests: urea breath test, rapid urease test (Pronto Dry), histology, and culture. Age, sex, gastroduodenal disease (duodenal ulcer, gastritis), observance of treatment, therapeutic regimen, sensitivity for antibiotics, smoking habit, and type of bacterial virulence were evaluated.

Results: The global rate of eradication for the four regimens was 74%. In multivariate analysis, risk factors of the failure of H. pylori infection treatment were inobservance of treatment (p = .01) and resistance to clarithromycin (p = .04) and metronidazole (p = .05).

Conclusion: In this study, inobservance of treatment and resistance to antibiotics, in particular to clarithromycin, were the two factors associated with the failure of H. pylori infection treatment.

Abstract no.: P03.06
Demographics of H. pylori Infection in a Canadian Arctic Hamlet

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In the predominantly Aboriginal Hamlet of Aklavik, Northwest Territories (population = 600), residents identified Helicobacter pylori infection and its link to gastric cancer as a priority concern and advocated for research focused on solutions. The resulting Aklavik H. pylori Project is the start of a broad collaboration aimed at investigating H. pylori infection in northern Canadian populations where gastric cancer rates are elevated and H. pylori infection is difficult to treat. Project goals are to describe sociodemographic patterns of H. pylori infection and the associated disease burden, identify effective treatments, inform local healthcare policy, and address community concerns. This report describes H. pylori prevalence in demographic subgroups. In January 2008 all Aklavik residents were invited to have a urea breath test (UBT) at the local health center. Of 368 residents who enrolled in the project, 313 were tested by UBT and 58% were positive. H. pylori prevalence was 61% in males (n = 140) and 56% in females (n = 173). In age groups 0–14, 15–24, 25–39, 40–59, and 60–79, prevalence was 53%, 70%, 69%, 51%, and 54% (n = 59, 53, 61, 105, 35), respectively. By ethnicity, prevalence was 65% in Inuvialuit (Inuit) (n = 157), 56% in Gwich’in Dene First Nation (n = 80), 67% in mixed/other aboriginal (n = 12), and 25% in nonaboriginals (n = 36) (missing ethnicity = 28). UBT screening showed that H. pylori prevalence in this Canadian Arctic Hamlet is high across aboriginal subgroups from an early age. This project will seek effective strategies for addressing community concerns about health risks from H. pylori infection in northern Canada.
Abstract no.: P03.07
Prevalence of H. pylori Infection and Upper Gastrointestinal Findings in Patients Undergoing Bariatric Surgery

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Background: Helicobacter pylori infection is present in 30–67% of patients scheduled for bariatric surgery. The role of upper gastrointestinal endoscopy (UGIE) before bariatric surgery is controversial.

Aim: To evaluate the prevalence of H. pylori infection and UGI findings in asymptomatic morbidly obese patients planning to undergo bariatric surgery.

Patients/Method: Between January 2007 and September 2008, all patients undergoing bariatric surgery underwent a routine UGIE with antral/fundus biopsies and H. pylori testing. The demographic, clinical, and endoscopic data were compared between H. pylori-positive and H. pylori-negative patients. H. pylori eradication was mandatory prior to surgery.

Results: Of the 284 patients that underwent bariatric surgery, 20.77% were male. One hundred and eight patients (38%) were H. pylori positive. The cohort average age was 42.18 years: 44 years versus 39 in H. pylori positive and H. pylori negative, respectively (p = .006). Two hundred and forty-four patients were Caucasian (85.92%) and 40 (14.08%) were non-Caucasian. H. pylori was more prevalent in non-Caucasian. The average body mass index (41 kg/m²) was similar in the two groups. For all patients, the most frequent endoscopic findings were hiatal hernia, esophagitis, and gastritis. All others were less frequent with Barrett esophagus in four patients, gastroduodenal ulcers in 31 patients, gastric polyps in eight patients, and silastic ring migration in six patients.

Conclusion: The prevalence of H. pylori in our patients is similar to the general population. This study showed a high incidence of endoscopic findings in asymptomatic obese patients. Systematic UGIE and H. pylori testing should be performed in all patients scheduled to undergo bariatric surgery.

Abstract no.: P03.08
Trend in the Eradication Rates of H. pylori Infection in the Last 11 Years

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Background/Aims: The standard triple therapy used as the first-line treatment for Helicobacter pylori that combines a proton pump inhibitor (PPI), amoxicillin, and clarithromycin had an initial eradication rate of 90%. However, many recent studies have not found this level of effectiveness. This study evaluated the trend in the eradication rates of H. pylori infection over the last 11 years.

Methods: This was a retrospective study of patients diagnosed with H. pylori infection between 1997 and 2007 and treated with triple therapy (PPI, amoxicillin, and clarithromycin). The patients answered questions about compliance and side-effects within 2 weeks of completing their treatment. In addition, we assessed whether the H. pylori had been eradicated at least 4 weeks after the treatment using a 13C-urea breath test, rapid urease test, or histopathologic examination.

Results: The eradication rate with first-line triple therapy decreased over the study period. There was no change in the eradication rate with second-line quadruple therapy (PPI, bismuth, metronidazole, and tetracycline). There were no differences in the eradication rate and recrudescence between 1- and 2-week regimens.

Conclusions: The effectiveness of the recommended first-line triple therapy for H. pylori eradication has decreased significantly in the last decade. Therefore, the first-line therapy based on the combination of PPI, amoxicillin, and clarithromycin may need to be changed in the near future.

Abstract no.: P03.09
The Prevalence and Structure of Peptic Ulcer Disease in Population of Tyva Republic

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Aim. To study the prevalence of peptic ulcer disease in Mongoloids and Europoids of Tyva.

Methods. Materials of endoscopic department of Tyva Republican Hospital for the 2005–2006 were analyzed. For this period gastroduodenoscopy was performed on 1861 adult Europoids (988 men, 873 women) and 5829 adult Tyvins (2802 men, 3027 women). IgG Helicobacter pylori and IgG Cag A H. pylori were determined by ELISA method in 424 Europoids and 316 Tyvins.

Results. The prevalence of peptic ulcer disease in Europoids was 13.7%, in Mongoloids – 6.6% (p < .001). The prevalence of duodenal ulcer in Europoids was 8.5% (10.9% in men; 5.7% in women); gastric ulcer – 5.2% (6.2% in men; 4.2% in women). The prevalence of duodenal ulcer in Tyvins was 3.5% (5.2% in men; 1.9% in women); gastric ulcer – 3.1% (4.3% in men; 2.0% in women). The prevalence of H. pylori was 92.4% in Mongoloids and 87.1% in Europoids. The frequency of Cag A H. pylori in Tyvins was 60.0%, in Europoids – 61.2%.

Conclusion. The differences of peptic ulcer prevalence in Mongoloids and Europoids of Tyva were registered, which were not associated with H. pylori prevalence.

Abstract no.: P03.10
Infection with H. pylori – Prevalence in Young Asymptomatic Volunteers

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Background and Objective: Recent data are lacking on the prevalence of Helicobacter pylori in young individuals in Germany.
It has been suggested that the age-adjusted prevalence of *H. pylori* decreased over the last decades in the younger generations. This study aimed to define the prevalence of *H. pylori* in young asymptomatic volunteers in mid-Germany.

**Subjects and Methods:** One hundred and thirty-seven healthy volunteers (age 18–40 years) were screened for *H. pylori* infection. *H. pylori* status was evaluated by 13C-urea breath test, stool antigen test, and *H. pylori* serology (IgG).

**Results:** Among the 137 persons tested (71 females, 66 males; mean age 27.14 ± 5.65), 15% were *H. pylori*-positive (20 of 137) by using either the breath or the stool antigen test. Sixteen of the 20 infected subjects had a positive result in both test methods. Three were only positive in the stool test, while one was positive only in the breath test. Considering a positive result in the *H. pylori* serology, the prevalence of a present or past infection increased to 26%. In the group aged 30–40 years the prevalence of *H. pylori* was higher (37%) compared to younger individuals (18–29 years) with 22%. In female participants the prevalence was 30% in comparison to 21% in male subjects.

**Conclusion:** Among young asymptomatic volunteers, there is still a remarkable prevalence of *H. pylori* infection, with the infection being more prevalent in female than male subjects. Interestingly, there is a difference in this young population between the prevalence of the current infection status (stool, breath test) and the serologic status. The known association between age and *H. pylori* infection was confirmed.

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**Abstract no.: P03.11**

**Prevalence of vacA Genotypes of *H. pylori* in Pakistani Population**

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*Helicobacter pylori* is a recognized cause of gastroduodenal diseases worldwide. Virulence of the organism is correlated with two virulence genes including cagA and vacA. Allelic variation is observed in signal sequence and middle region of vacA gene which is found to be different in different geographic areas. In Pakistan, studies about prevalence and genomic diversity of *H. pylori* are largely missing. This study was designed to know prevalence of *H. pylori* and its vacA genotypes in metropolitan city of Karachi, Pakistan. A total of 375 gastric biopsies of the patients with various gastroduodenal symptoms were included in the study. Genomic DNA were purified and characterized for 16s rRNA, ureA, and vacA genotyping using polymerase chain reaction. Of 375 patients, 44.5% were found to be infected with *H. pylori* using rRNA-specific primers. Thirty-nine percent were positive using ureA-specific primers. The vacA s1a-m1 genotype was most prevalent and observed in 50% cases followed by s1a-m2 in 21% and s2-m1 in 14.5% cases. Genotypes s1b-m1 and s1b-m2 were observed in 3% cases each. No s2-m2 genotype was observed. Our observations regarding s1-m1 and s1-m2 genotypes are in consensus with previous studies conducted in Asian countries; however, prevalence of genotype s2-m1 is higher (14%) in Pakistani population. To the best of our knowledge, this is first comprehensive study about prevalence of *H. pylori* in Pakistan with considerable number of samples which also provide molecular dissection of vacA gene.

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**Abstract no.: P03.12**

**Omeprazole Permeabilizes Yeast to Release Intracellular *H. pylori***

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**Introduction:** Omeprazole is a proton pump inhibitor which exerts its inhibitory effect on ATPase of eukaryotic cells. Accordingly, omeprazole exhibits antifungal activity against yeasts. In this study we treated yeasts with acid-activated omeprazole and examined the release of bacterium-like bodies (BLBs) outside the yeasts.

**Methods:** One oral yeast, *Candida* spp., was selected for the study. Polymerase chain reaction was performed by designed primers to amplify *H. pylori* 16S rDNA and jhp0947 genes from the total DNA extracted from yeast. Yeast suspension was prepared with the turbidity of 0.5 McFarland in BHI broth. A concentrated stock solution of omeprazole was prepared in DMSO then acidified to pH 2 with 0.1 mol/L HCl. Ten microlitres of omeprazole was added to 990 µL of yeast suspension then incubated at 35 °C for 24 hours. Wet mount was prepared and examined by light microscopy.

**Results:** Electrophoresis demonstrated the amplified 16S rDNA (519 bp) and jhp0947 (611 bp) genes from yeast DNA. The size of the products was homologous to the ones amplified from control *H. pylori*. Microscopic observations demonstrated the presence of BLBs inside and outside of yeasts. Attempts to culture the BLBs were not successful.

**Discussion:** Previous microscopic observations of gastric yeasts have revealed the presence of intracellular BLBs inside the yeast vacuole. Attempts to release or culture bacteria from yeasts have not been successful until now. Here treatment of yeasts with omeprazole led to release of BLBs which were not culturable. Amplification of *H. pylori*-specific genes from yeast propose that BLBs might have bacterial nature and could be *H. pylori*.

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**Abstract no.: P03.13**

**Detection of *H. pylori* in the Vacuole of Yeast by Live/Dead Bacterial Viability Kit**

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**Introduction:** Yeasts have vacuole for storage of phosphate and amino acids. Bacteria could establish in this vacuole to be...
protected against environmental stresses, while being able to reach important nutrients. In this study intracellular existence of Helicobacter pylori inside the yeast vacuole was examined by polymerase chain reaction (PCR) and live/dead kit.

**Materials and Methods:** One oral yeast Candida was subcultured 10 times on yeast glucose chloramphenicol agar for the elimination of possible bacterial contamination. PCR was performed to amplify H. pylori 16S rDNA and jhp0947 genes from the total DNA of yeast. Live/Dead BacLight Bacterial Viability Kit was used for the assessment of living status of intracellular H. pylori inside the vacuole. Spiral and coccoid forms of H. pylori were also recruited as controls.

**Results:** H. pylori-specific genes, 16S rDNA and jhp0947, were amplified from the total DNA of yeast. The size of amplified fragments was homologous to the ones amplified from control H. pylori. Fluorescent microscopic observations of stained wet mount of yeast revealed fast moving green fluorescent bacterium-like bodies inside the vacuole. Most of the coccoid and spiral forms of H. pylori appeared green.

**Discussion:** Results of this study propose the possibility of endosymbiotic life of H. pylori inside yeast. Candida yeast and H. pylori are both colonizers of human digestive tract. Thus their intimate relationship might have an evolutionary rationale. Since yeast is remarkably compatible with environmental changes, establishment of bacteria inside its vacuole could be very crucial for the protection of bacteria in nature. Advantage of this relationship for yeast has to be elucidated.

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**Abstract no.: P03.14**

**Helicobacter Infection in Cats of the North of Portugal Preliminary Results**

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**Introduction:** Gastric helicobacter-like organisms (GHLOs) were described in humans, domestic, and wild animals: 32 species have been identified and mixed infections can occur. Human represents the main reservoir of H. pylori, with a prevalence of around 50% in developed countries and up to 90% in Third World countries. Portugal, with 83%, has the highest rates in Europe. In 2008, 18.4% in developed countries and up to 90% in Third World countries. Portugal, with 83%, has the highest rates in Europe. In 2008, 18.4% of cats brought to ICBAS-UP Animal Hospital presented gastrointestinal disorders as main complaint. In order to determine the presence of GHLOs in cats of northern Portugal, endoscopic examina-

**Material and Methods:** Gastric endoscopy was performed in nine cats, seven with gastrointestinal signs. Biopsies of cardia, stomach body, and antrum were collected, routinely processed for histopathology, and stained with hematoxylin and eosin, modified Giemsa stain, and immunohistochemistry.

**Results:** All cases revealed the presence of GHLOs, most without associated inflammatory reaction. Sensitivity increases from hematoxylin and eosin to histochemistry being higher in immunohistochemistry. Strains identification and isolation are underway.

**Conclusion:** A clear association between infection by GHLOs, gastritis, and gastric malignancies is already documented. The fact that some infected cats show no clinical signs, means that this condition is underdiagnosed. Northern Portugal has a higher incidence of H. pylori in humans and the detection of this bacteria in cats, in the same niche, may indicate that these animals represent a silent reservoir. The public health repercussions of this fact emphasize the need to determine the prevalence of different GHLOs in this area.

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**Abstract no.: P03.15**

**H. pylori Colonization of the Adenotonsillar Tissue: A Literature Review**

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Recently, several studies have evaluated the presence of Helicobacter pylori in the adenotonsillar tissue. The present study aimed at critically reviewing the evidence so far obtained in those studies. For that, a PubMed search with keywords related to adenoid and tonsillar tissues and H. pylori was performed. Studies were analyzed regarding the total number of patients and number of positive results with each methodology.

Several techniques have been used, isolated, or in combination, to test for the presence of H. pylori. Positive results where found in nine of the 11 papers that have used the rapid urease test (158 of 477 patients). Positivity was obtained in one of three studies that have used histology (four of 104 patients), whereas immunohistochemistry with antibodies to H. pylori gave positive results in two of five papers analyzed (30 of 342 patients). Polymerase chain reaction was used in eight studies, and in five of them positivity for H. pylori was reported (61 of 303 patients). Only four studies evaluated the presence of H. pylori by culture and only in one of them positive results were obtained for gram-negative, catalase, oxidase, and urease-positive bacteria (61 of 303 patients).

In conclusion, the review of these publications demonstrated contradictory results. The reasons underlying these observations may be related to differences in sensitivity and specificity of methods. Techniques currently used for detecting gastric H. pylori colonization are probably not adequate to evaluate infection of the adenotonsillar tissue.

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**Abstract no.: P03.16**

**H. pylori Infection in the Countries of the Caribbean**

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**Objective:** Our aim was to determine the seroprevalence of Helicobacter pylori infection in a group of 300 consecutive adult subjects population submitted to upper digestive tract endoscopy clinics in three countries, Venezuela, Cuba, and Dominican Republic.

**Subjects and Methods:** Serology (IgG) were performed on 300 patients using Microwell ELISA from Diagnostic Automation Inc. (USA) and Pyloriset E IA-IIIG de Orion Diagnostic (Finland). Patients had the following endoscopic diagnosis: duodenal ulcer 31 of 300 (10%); gastric ulcer: 27 of 300 (9%); and nonulcer dyspepsia, including chronic gastritis: 242 of 300 (81%). The mean age was 46 years with 127 of 300 (42%) men and 173 of 300 (58%) women.

**Results:** Among the 300 serums tested, 100% were positive in Venezuela, Cuba, and Dominican Republic. The seroprevalence
of *Helicobacter pylori* infection in the symptomatic population of La Havana-Cuba, Caracas-Venezuela, and Santo Domingo-República Dominicana.

Conclusions: There is a great paucity of information about *H. pylori* infection in the countries of the Caribbean basin. These results indicate the importance of further studies to identify factors influencing the high prevalence of the infection with *H. pylori* in the region.

**Abstract no.: P03.17**
Identification of Eastern Asian cagA 3′ Region Using a Single PCR Technique

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CagA of *Helicobacter pylori* is a virulence factor and part of the cag PAI. It encodes a protein which is 120–140 kDa and immuno-

**P04 Inflammation, Host response, Immunity, Animal Models, and Vaccines**

**Abstract no.: P04.01**
The *H. pylori*-Induced Reduction of Secretory Leukocyte Protease Inhibitor Protein Levels is Regulated by Post-translational Mechanisms in a CagA-Independent Manner

T. Wex,* † D. Schindele,* † A. Krieg,* † U. Peitz,* M. Naumann† and P. Malfertheiner*†

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Background: Recently, a downregulation of mucosal secretory leukocyte protease inhibitor (SLPI) expression by *H. pylori* was reported. Here, the regulatory mechanisms of SLPI expression were studied in gastric tumor cells infected with *H. pylori* and isogenic mutants.

Methods: Gastric tumor cells (AGS and MKN-28) were infected with *H. pylori* CagA+ strain and isogenic mutants lacking either functional CagA or T4SS. Furthermore, the effects of cycloheximide, chloramphenicol (as inhibitors for protein translation), and the direct cell–bacteria contact were studied in context to the *H. pylori*-induced reduction of SLPI. SLPI gene expression was studied by ELISA and quantitative RT-PCR.

Results: Gastric cell lines infected by wild-type *H. pylori* showed a reduction by 30–80% of SLPI protein levels, but a 5-fold induction of corresponding transcript expression (*p < .001*). The reduction of the SLPI protein amount was independent of direct cell–bacteria interaction, *and* does not depend on *de novo* protein biosynthesis in bacteria. Data from clinical specimens and in vitro studies using *H. pylori* mutants lacking either CagA or VirB7 revealed that the reduction of the SLPI is independent of CagA and the presence of a functional T4SS. Coincubation studies with cycloheximide demonstrated that *H. pylori* specifically induces post-translational processes leading to the reduction of SLPI. Initial studies targeting cathepsins and metalloproteases as SLPI-degrading proteases failed to demonstrate an involvement of these enzymes.

Conclusion: The *H. pylori*-induced reduction of epithelial SLPI protein is mainly regulated by post-translational processes, and does not depend on bacterial virulence factor CagA and the presence of T4SS.

**Abstract no.: P04.02**
In vitro Study of Dendritic Cell Maturation Induced by *H. pylori* Strains: Evaluation of the Inflammatory Response and Immunologic Consequences

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In the context of gastric MALT lymphoma, our aim was to investigate ex vivo the role of dendritic cells (DC) in response to...
of *H. pylori* infection in the symptomatic population of La Havana-Cuba, Caracas-Venezuela, and Santo Domingo-República Dominicana.

**Conclusions:** There is a great paucity of information about *H. pylori* infection in the countries of the Caribbean basin. These results indicate the importance of further studies to identify factors influencing the high prevalence of the infection with *H. pylori* in the region.

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CagA of *Helicobacter pylori* is a virulence factor and part of the cag PAI. It encodes a protein which is 120–140 kDa and immuno-
dominant antigen. There are two main CagA polymorphisms in the 3′ region of *H. pylori* genome. One is specific of *H. pylori* strains of Eastern Asian origin and the other one is of Western origin. Differences in polymorphisms are documented by DNA and amino acid sequence analysis. The goal of the present study was to identify strains of East Asian origin by polymerase chain reaction (PCR) technique.

**Method:** A total of 120 *H. pylori* strains were studied. There were 60 isolated from patients of Western origin and 60 were obtained from patients of East Asian origin. We performed two Eastern-specific PCR that amplified a band in the cagA region before and after the D motif.

**Results:** We found that both sets of primers had a high specificity since only 5% of none Eastern *H. pylori* showed some PCR bands. In contrast, the sensitivity was better for the PCR amplifying the cagA gene region before the D motif (83%) than the PCR amplifying the region of the cagA gene that included a region after the D motif (63%). Sequence analysis failed to show differences in nucleotide sequences that explain the discordant results.

**Conclusion:** We have designed a PCR that is highly specific and sensitive to identify *H. pylori* that contains cagA gene harboring the specific Eastern D EPIYA motif.

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**P04 Inflammation, Host response, Immunity, Animal Models, and Vaccines**

**Abstract no.: P04.01**

**The *H. pylori*-Induced Reduction of Secretory Leukocyte Protease Inhibitor Protein Levels is Regulated by Post-translational Mechanisms in a CagA-Independent Manner**

T. Wex,* D. Schindele,* A. Krieg,* U. Peitz,*
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**Background:** Recently, a downregulation of mucosal secretory leukocyte protease inhibitor (SLPI) expression by *H. pylori* was reported. Here, the regulatory mechanisms of SLPI expression were studied in gastric tumor cells infected with *H. pylori* and isogenic mutants.

**Methods:** Gastric tumor cells (AGS and MKN-28) were infected with *H. pylori* CagA+ strain and isogenic mutants lacking either functional CagA or T4SS. Furthermore, the effects of cycloheximide, chloramphenicol (as inhibitors for protein translation), and the direct cell–bacteria contact were studied in context to the *H. pylori*-induced reduction of SLPI. SLPI gene expression was studied by ELISA and quantitative RT-PCR.

**Results:** Gastric cell lines infected by wild-type *H. pylori* showed a reduction by 30–80% of SLPI protein levels, but a 5-fold induction of corresponding transcript expression (*p < .001*). The reduction of the SLPI protein amount was independent of direct cell–bacteria interaction, and does not depend on de novo protein biosynthesis in bacteria. Data from clinical specimens and in vitro studies using *H. pylori* mutants lacking either CagA or VirB7 revealed that the reduction of the SLPI is independent of CagA and the presence of a functional T4SS. Coincubation studies with cycloheximide demonstrated that *H. pylori* specifically induces post-translational processes leading to the reduction of SLPI. Initial studies targeting cathepsins and metalloproteases as SLPI-degrading proteases failed to demonstrate an involvement of these enzymes.

**Conclusion:** The *H. pylori*-induced reduction of epithelial SLPI protein is mainly regulated by post-translational processes, and does not depend on bacterial virulence factor CagA and the presence of T4SS.

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**Abstract no.: P04.02**

**In vitro Study of Dendritic Cell Maturation Induced by *H. pylori* Strains: Evaluation of the Inflammatory Response and Immunologic Consequences**

I. Kaafarany,* C. Staedel,† V. Pitard,‡ P. Blanco,‡
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In the context of gastric MALT lymphoma, our aim was to investigate ex vivo the role of dendritic cells (DC) in response to
Helicobacter pylori, by studying cytokine production and microRNA (miRNA) expression.

Human DCs were matured in the presence of interleukin (IL)-4 and granulocytre microphage colony-stimulating factor (GM-CSF), and thereafter cocultured in the presence of H. pylori strains isolated from low grade gastric MALT lymphoma or duodenal ulcer patients. DC surface maturation markers were determined by flow cytometry, and secreted cytokines by antibody array and ELISA. The ability of H. pylori-activated DCs to induce allogeneic T lymphocyte proliferation was measured by bromodeoxyuridine incorporation and CD3 expression. DC expression of several miRNAs was determined total RNAs by quantitative RT-PCR.

Four gastric MALT lymphoma and 10 duodenal ulcer H. pylori strains were tested on DCs. A significant expression was obtained for each maturation marker molecules. All H. pylori strains were able to induce the production of several chemokines such as ENA-78, MIP1-delta, MCP-1, GRO, GRO-alpha, as well as the cytokines GM-CSF, tumor necrosis factor-alpha, IL-6, IL-7, and IL-10. A tendency was observed for IL-23 which was induced more by gastric MALT lymphoma strains than by duodenal ulcer strains. High induction of miR-155, and miR-146 was also observed. Finally, H. pylori-activated DCs were able to induce a significant T lymphocyte proliferation.

Our results show that H. pylori was able to activate DCs ex vivo, thereby promoting T lymphocyte proliferation. H. pylori is able to induce several miRNAs that have been implicated in pathologies such as lymphoma and cancers.

Abstract no.: P04.03
H. pylori Induce Interferon Gamma Production from Human NK Cells via TLR2

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Helicobacter pylori induces a chronic inflammation in the human gastric mucosa characterized by increased production of interferon (IFN)-gamma. The presence of natural killer (NK) cells in the human gastric mucosa and the ability of NK cells to produce IFN-gamma suggest an important role of NK cells in the immune response directed towards H. pylori infection. Since NK cells previously have been shown to respond to bacterial components with IFN-gamma production, we investigated the mechanisms for the recognition of H. pylori. Initial results indicate an involvement of Toll-like receptors (TLRs), and in particular TLR2.

To further confirm involvement of TLR signaling in the recognition of H. pylori MyD88 homodimerization was inhibited which resulted in decreased production of IFN-gamma, and inhibition of the p38 MAPK decreased the production as well as the secretion of IFN-gamma.

Furthermore, the H. pylori-specific putative lipoprotein HpaA was able to induce IFN-gamma production in NK cells, possibly via TLR2.

In conclusion, we suggest an involvement of TLR2 in the recognition of H. pylori by human NK cells and that HpaA might be important for recognition of the bacterium.

Abstract no.: P04.04
Helicobacter Flagellins Evade Physical Binding to TLR5, but Bind to Epithelial Cells and Influence Cellular Signaling

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Flagellins of the chronic gastric pathogen Helicobacter pylori and related bacteria have been shown to deploy only low potential to activate proinflammatory signaling in gastric and intestinal epithelial cells, which possess active Toll-like receptor 5 (TLR5). Flagellins of numerous other pathogenic and nonpathogenic bacterial species are potent activators of the human innate immune system and of proinflammatory responses by binding to TLR5. Evasion of TLR5-mediated responses appears to be a potent strategy for persistent bacteria such as H. pylori to evade the human innate and adaptive immune reaction, and to possibly downmodulate cancer-protective responses. The mechanism of the flagellar immune evasion by H. pylori was not known.

We show here by coprecipitation and binding assays that both H. pylori flagellins, FlaA and FlaB, which both have low activity to induce NF-kB activation and IL-8 release via TLR5, do evade stable physical binding to TLR5, but still bind to human and mouse cells. Moreover, H. pylori flagellins can induce extensive signaling in gastric epithelial cells as assessed by microarray hybridization and RT PCR. In order to assess the contribution of TLR5 to these signaling events, primary mouse embryonal fibroblasts (MEF) of TLR5−/− mice were employed. Activation assays of primary MEF and comparative microarray hybridization showed that regulation events induced by H. pylori flagellins are predominantly independent of TLR5. These results, which will be discussed further in the context of novel proposed intracellular flagellin sensors (Nod-like receptors), show that cellular signaling induced by Helicobacter flagellins can function independently of TLR5.

Abstract no.: P04.05
Mathematical Model of Complement-Mediated Bactericidal Activity of Human Serum Against H. pylori

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Introduction: Complement system (CS) is able to inactive bacteria due to pores formation in their membranes followed by osmotic lysis of bacterial cells. The aim of this study was the development of mathematical model of dynamic interaction of Helicobacter pylori and human serum contained anti-H. pylori antibodies, microbiologic study of this interaction, and comparison of theoretical and experimental results.
**Methods:** *H. pylori* NCTC 11639 and CCBH 642 freshly isolated from antral mucosa of patient with duodenal ulcer were used. Serum of the same patient was used also. Optically standardized suspensions of both strains cultures were mixed in equal volumes with undiluted serum and serum diluted to 1:2, 1:5, and 1:10. PBS and heat-inactivated serum were used as controls. The model was developed as the system of nonlinear first order ordinary differential equations.

**Results:** High level of anti-*H. pylori* antibodies in serum was obtained. Hemolytic activity of undiluted and diluted to 1:10 serum was estimated as 1:64 and 1:16, respectively. Undiluted serum and all its dilutions completely inactivated bacterial cells of homologous strain *H. pylori CCBH 642*. Undiluted serum and its dilutions up to 1:5 completely inactivated bacterial cells of reference strain *H. pylori* NCTC 11639. Serum diluted to 1:10 significantly (*p < .05*) inactivated *H. pylori* NCTC 11639 from 5.48 to 2.15 (log CFU/mL). Nonlinear mathematical model of dynamic of CS activation by *H. pylori* was proposed.

**Conclusion:** CS is able to inactivate *H. pylori* in vitro. Mathematical model results are in good agreement with experimental data.

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**Abstract no.: P04.06**

**Distribution of *H. pylori* Genotypes, Polymorphic Loci of Cytokine Genes (*IL-1* and *IL-10*), and Ulcer Sizes in Patients with Duodenal Ulcer Disease in Kazan, Russia**

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**Aim:** The aim of our work is to analyze the distribution of *Helicobacter pylori* genotypes (cagA, vacAs1/s2), polymorphic loci of interleukin (*IL*)-1 and -10 cytokines’ genes (*IL-1*-511>C>T, *IL-1B*-3954>C>T, *IL-1RN* (VNTR), *IL-10* (*IL-10*-1082>G>A) as well as morphometric characteristics of ulcers in patients with duodenal ulcer disease in Kazan, Russia.

**Material and Methods:** Genomic DNA of patients (n = 88) and healthy individuals (n = 123) was used for the study. Polymerase chain reaction and restriction fragment length polymorphism were used for genotyping of *IL-1* and *IL-10* in samples. The presence of bacterial virulence genes was investigated by *cagA, vacAs1/s2* PCR. All PCR products were analyzed by electrophoresis in 2% agarose gel stained with ethidium bromide.

**Results:** We found that most of patients (61%) were infected with high-virulent *H. pylori* strains — *cagA* vacAs1/s2*. Individuals with *IL-1B*-511>C allele and *IL-1B*-511>C>C genotype have an increased risk of *H. pylori*-associated duodenal ulcer disease development [odds ratio (OR) = 2.07, 95% confidence interval (CI) 1.37–3.14; OR = 4.56, 95% CI 2.18–9.56, respectively], whereas those with *IL-1B*-511>C allele, *IL-1B*-511>C>C and *IL-1RN*<1/2 genotypes have the decreased one (OR = 0.48, 95% CI 0.32–0.73; OR = 0.47, 95% CI 0.26–0.85; OR = 0.47, 95% CI 0.26–0.84, respectively). We observed that patients with *IL-1RN*<1/2 genotype or infected with *H. pylori cagA* vacAs1/s2 strains have the low probability for spontaneous healing of the ulcers (OR = 0.05, 95% CI 0.003–0.90; OR = 0.27, 95% CI 0.10–0.74, respectively).

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**Abstract no.: P04.08**

**Rapid Internalization of *H. pylori* into AGS Cell Line**

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The infection of host cells by *Helicobacter pylori* is quite complex and incompletely understood. *H. pylori* has been described as a noninvasive bacterium, but there is a growing body of evidence that this bacterium may in fact be an intracellular organism. Several studies have shown that *H. pylori* is capable of repopulating the extracellular environment after gentamycin eradication. The infection by *H. pylori* also appears to trigger apoptosis via interaction with death receptors in the plasma membrane activating mitochondrial apoptosis.

To search for intracellular *H. pylori*, we used transmission electron microscopy (TEM) after coculture of human gastric...
epithelial cells (AGS) with strain 1713. The H. pylori strain 1713 was isolated from a patient presenting normal gastric mucosa. Seven replicates of the coculture with a multiplicity of infection of 100:1 were performed. After incubation for 0, 0.5, 1, 2, 6, 12, and 24 hours, samples were collected for TEM analysis. Microbial adherence to epithelial cell was detected after 30 minutes, and internalized H. pylori cells after 1 hour of incubation, together with apoptotic cells. Large intracellular vacuoles containing bacteria were detected after 24 hours of incubation. This work supports the hypothesis that the life cycle of H. pylori may include an intracellular step that may protect the bacteria from the hostile gastric environment and explain antibacterial therapy failure. Gastric epithelial cells may serve as a reservoir within a reservoir (human stomach). Apoptosis is induced early in the interaction of extracellular bacteria with the cells and may be important for pathogenesis mechanism.

Abstract no.: P04.09
Differential Mucosal Activity of Two Superoxide Dismutase Isoforms in Chronic Gastritis
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Introduction: The involvement of reactive oxygen species in the inflammatory tissue destruction is well established. The human gastric diseases have shown significant changes in the activity and expression of superoxide dismutase isofoms (SOD).
Aim: The aim of this study is to detect Mn-SOD and CuZn-SOD activity as well as their expression in gastric tissue homogenate and to evaluate their relationship to pathohistologic diagnosis.
Materials and Methods: In this study, 30 patients with gastritis (chronic and chronic active) were recruited. Patients with intestinal metaplasia, carcinoma cells, and lymphoma cells were excluded from the study. Biopsy specimens (60 samples) were obtained from the gastric antrum and corpus of all patients. According to the updated Sydney System H. pylori, neutrophil and mononuclear cell infiltration, atrophy and intestinal metaplasia were graded semiquantitatively as absent, mild, moderate, and marked. The diagnosis of H. pylori infection was detected by immunohistochemical method. Gastric tissue samples were used to determine activity and expression of Mn-SOD and CuZn-SOD by spectrophotometric analysis and confirmed with anti-Mn-SOD Ab immunohistochemically.
Results: Two SOD isoforms were found to be differentially affected in inflamed gastric mucosa. Enzyme activity measurements showed consistent results for Cu, Zn-SOD, but Mn-SOD was hardly affected by the severity of inflammation.
Conclusions: These results suggest that an evaluation of tissue Mn-SOD activities in gastritis is useful factor for estimating the importance of chronic inflammation.

Abstract no.: P04.10
Immunomodulatory Activity of H. pylori LPS – Possible Reason of Chronic Infections
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Background: Helicobacter pylori causes chronic duodenal diseases. An outcome of infections depends on the host innate and adaptive immune response. H. pylori lipopolysaccharide (LPS) has an immunomodulatory potential.
Aim: We asked whether H. pylori LPS influence a cytotoxic and proliferative activity of human peripheral blood mononuclear leukocytes (MNC) from H. pylori infected (Hp+) or uninfected (Hp–) donors.
Methods: Proliferation was estimated in 5 days MNC cultures with or without H. pylori LPS. Blastogenesis was determined by incorporation of [3H]thymidine. Cytotoxic activity of lymphocytes, unstimulated or stimulated with LPS, towards HeLa cells was determined by MTT reduction, MitoLight assay (Chemicon) was used to detect apoptosis.
Results: Lymphocytes from Hp+ donors, stimulated with H. pylori LPS, had lower cytotoxic activity as compared to uninfected individuals. Such activity showed 79% (15 of 19) and 50% (5 of 10) of lymphocytes from Hp– and Hp+ donors, respectively. Lymphocytes from four of 19 Hp– donors were killing target cells with the efficacy over 20%. The cells of Hp+ donors did not show such high cytotoxic activity. The Hp LPS was a poor stimulator of MNC proliferation. Only three of 10 Hp+ and two of 11 Hp– MNC proliferated in the presence of H. pylori LPS. The spontaneous proliferation of the majority of MNC cultures was inhibited in the presence of H. pylori LPS. This was correlated with the apoptosis symptoms.
Conclusions: Lymphocytes from Hp+ donors generate less robust cytotoxic activity as compared to Hp– individuals in response to H. pylori LPS. The H. pylori LPS impairs the lymphocyte proliferation. These two phenomena may help to explain the chronic character of H. pylori infections.

Abstract no.: P04.11
Association of CagA H. pylori with Apoptosis in Gastric Antral Mucosa in Gastritis Patients Among Inhabitants of Eastern Siberia
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Aim: To study apoptosis parameters in gastric antrum mucosa in dependence on CagA Helicobacter pylori in patients with chronic gastritis.
Methods: We examined 23 Evenks and 24 Europoids with histologically confirmed gastritis aged 18 to 50 years living in...
Abstract no.: P04.12
Regulation of Host Cell miRNA by H. pylori in the Context of Innate Immunity

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MicroRNAs (miRNAs) constitute an abundant class of small noncoding RNAs that regulate protein expression by either mRNA degradation or translational inhibition. miRNAs are involved in many physiologic processes including cellular development, but also in pathologic processes like cancerogenesis.

H. pylori is a human pathogen living in the stomach of around 50% of the world's population. Infection with H. pylori evokes an innate immune response in epithelial cells, macrophages, and other cell types. H. pylori induces signals mainly via the Toll-like receptor (TLR) family as well as the nucleotide oligomerization domain (NOD) receptors, thereby releasing proinflammatory cytokines. We are interested in the innate immune response of H. pylori with respect to the expression of miRNAs during this process. By performing miRNA microarray, Northern blot, and realtime-RT-PCR experiments we determined one particular miRNA to be upregulated after H. pylori infection in the murine macrophage cell line J774.A as well as primary macrophages (BMDMs). The investigated responses showed a strong dependence on extracellular TLRs as well as intracellular receptor signaling.

Abstract no.: P04.13
Association of Apoptosis in Gastric Mucosa and Antral Atrophy in Patients with Chronic Gastritis Among Inhabitants of Eastern Siberia

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Aim: To study apoptosis parameters in gastric antrum mucosa in dependence on presence of gastric atrophy in patients with chronic gastritis.

Methods: We examined inhabitants of Evenkia (23 Evenks, 24 Europoids) with histologically confirmed gastritis in age 18–50 years. All subjects underwent esophagogastroduodenoscopy, and antrum mucosa biopsy specimens were taken. Morphologic research included microscopic examination after staining by hematoxylin and eosin with description of results using visual analog scale (Dixon et al., 1996) and definition of H. pylori dissemination after Gimsa staining. Apoptosis in gastric antrum mucosa was determined by TUNEL method. Apoptotic index (AI) was determined by counting percentage of TUNEL-positive cells at ×400 magnification. Statistical analysis was performed using the Spearman's rank correlation coefficient (r).

Results: In gastric antrum among Europoids with CagA H. pylori in superficial epithelium AI was – 3.41 ± 0.33%, in patients without CagA – 2.57 ± 0.24% (p = .09, r = 0.27), in glandular epithelium – 4.56 ± 0.51% and 3.93 ± 0.54% (p = .21, r = 0.20), in stroma – 7.80 ± 0.89% and 5.51 ± 0.62% (p = .03, r = 0.34) accordingly. The summary apoptotic index in antral mucosa in CagA-positive alien inhabitants was 6.06 ± 0.58%, in CagA-negative persons – 4.22 ± 0.39% (p = .01, r = 0.39).

Among Evenks with CagA H. pylori in superficial epithelium AI was 1.81 ± 0.17%, in glandular epithelium 3.01 ± 0.16%, in stroma 5.71 ± 0.46%, summary AI – 4.23 ± 0.34%. In CagA-negative native inhabitants AI was – 1.64 ± 0.19% (p = .71, r = 0.07), 2.14 ± 0.16% (p < .001, r = 0.59), 3.57 ± 0.46% (p < .001, r = 0.64), and 2.95 ± 0.30% (p < .001, r = 0.64) accordingly.

Conclusion: In gastric antrum the correlation of apoptosis with CagA H. pylori was higher in Evenks in comparison with Europoids.

Abstract no.: P04.14
Atherosclerosis and Coronary Heart Disease – Correlation with H. pylori and Chlamydophila pneumoniae Infections

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Background: Human atherosclerosis is an inflammatory disease probably linked to Helicobacter pylori and Chlamydophila pneumoniae
infections. Autoimmune reactions are an important aspect of coronary heart disease (CHD).

**Objectives:** The prevalence and the levels of IgG recognizing *H. pylori* and *C. pneumoniae* antigens or mycobacterial heat shock protein (Hsp65) and human Hsp60 in the patients with CHD, dyspeptic patients (DP), and healthy subjects (HI) were estimated. The frequency and the levels of anti-*H. pylori* and *C. pneumoniae*-IgA were determined.

**Methods:** ELISA was conducted with glycine acid extract (GE) from the reference *H. pylori* strain, chlamydial lipopolysaccharide, mycobacterial Hsp65, and human Hsp60. Antibodies to classified *H. pylori* antigens were detected by Western blot.

**Results:** IgG to GE were found with the frequency: 92%, 100%, and 43% in CHD, DP, and HI group, respectively, p < .05, whereas IgG, to GE was detected with higher frequency and intensity in CHD and DP than in HI group, p < .05. CHD patients had higher anti-GE IgA then DP and HI group, p < .05. The prevalence of anti-*C. pneumoniae* IgG was higher in CHD and DP than in HI group, p < .05. In CHD patients the production of anti-*C. pneumoniae* IgA was more prevalent than in DP – 28% and HI – 52%, p < .05. Anti-*H. pylori* Hsp60 IgG were detected for 84% of CHD, 90% of DP, and 66% of HI group. IgG to Hsp65 were produced by 62% of CHD, 60% of *H. pylori* infected, 56% of HI, and 70% of tuberculosis patients, p > .05.

**Conclusions:** Chronic *H. pylori* and chlamydial infections are typical for CHD patients. Enhanced production of antibodies to such pathogens may influence atherosclerosis.

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**Abstract no.: P04.15**

**Polymorphisms in Genes Interleukins Associated with Ulcer Disease in a High-Risk Khakas Population**

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**Aim:** To reveal possible association of spreading polymorphisms in genes C+3953T IL1B, VNTR IL1RN, T−251A IL8 and *H. pylori*−dependent ulcer disease (UD).

**Materials and Methods:** DNA from 25 unrelated Khakas Mongoloids patients with UD and 123 ethnically matched healthy controls was typed for the IL1B +3953, IL8 –251 gene polymorphisms, and the VNTR polymorphism in intron 2 of the IL1RN gene by polymerase chain reaction (PCR)-based methods. *H. pylori* status was determined in all patients and controls.

**Results:** Within the predominant genotype, which was determined in the groups being investigated, were R4/R4 IL1RN (UD – 76.0%, in the control – 79.6%, p > .05) and CC +3953 IL1B (UD – 84.0%, in control – 73.2%, p > .05). The genotype AA –251 IL8 was a genetic risk factor for UD in patients with *H. pylori* infection (odds ratio = 3.09, 95%, confidence interval 1.08–8.77).

**Conclusion:** Our results provide further evidence that host genetic factors play a key role in the pathogenesis of *H. pylori*−dependent UD. Technological platform for molecular medicine of *H. pylori*−associated diseases is based on identification of molecular and genetic mechanisms and ethnic peculiarities of interactions between the pathogen and the human organism.

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**Abstract no.: P04.16**

**The Enhancement of Gastric Cell Lines Transfection by Nanoparticles for Oral Vaccine Strategies**

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Mucosal vaccination against *Helicobacter pylori* may induce immune response and prevent infection only when powerful mucosal adjuvants are used along with the antigens. As adjuvants such as cholera toxin or heat-labile toxin of *Escherichia coli* cannot be used for humans for their toxicity, finding nontoxic alternative adjuvants or immunization strategies is crucial for the development of efficacious mucosal *Helicobacter* vaccines.

Biodegradable nanoparticles have been investigated as vaccine delivery systems for protein antigens. Furthermore, administration of naked DNA has shown promising results for vaccination, little success has been achieved after oral delivery. This work combines these two promising approaches, focusing on the oral delivery of plasmid DNA using biocompatible and mucoadhesive chitosan nanoparticles. To overcome the genetic variability of *H. pylori* a plasmid DNA vaccine, containing fragments of the immunogenic vacA, groEL, and homp antigens, was constructed. Each 50 aminoacid-long fragment represents the most conserved and immunogenic region among different *H. pylori* strains. The immunogenicity for B and T epitopes was determined by the Jameson and Wolf, the AMPHI, and the Rothbard–Taylor methods.

Chitosan nanoparticles carrying plasmid DNA were successfully produced and physicochemically characterized using biochemical, microscopic, and light-scattering techniques. Stability of nanocapsulated DNA was studied in simulated gastric and simulated intestinal media at 37 °C. Released DNA was evaluated by agarose gel electrophoresis.

In vitro transfection efficacy was evaluated under different culture conditions using MKN45, N87, and AGS human gastric epithelial cell lines with distinct cell differentiation.

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**Abstract no.: P04.17**

**The Role of Cathepsin X in the Immune Response to the Infection with *H. pylori***

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Cathepsin X has been shown to regulate immune response. Its active role has been demonstrated previously in chronic inflammation of gastric mucosa and tumorigenesis of gastric cancer. In this study we demonstrate its role in presenting the antigens to immune cells and in the immune response to *Helicobacter pylori* infection.

We used the monoclonal antibody against cathepsin X and flow cytometry to determine the level of cathepsin X in THP-1 cells primed with *H. pylori* antigens isolated from subjects suffering
The 13C-urea breath test (UBT) is sensitive and specific for detection of human infection with *H. pylori*. The aim of the present study was to validate the use of UBT for follow-up of this infection on animal models of gastric infection.

**Aim:** The 13C-urea breath test (UBT) is sensitive and specific for detection of human infection with *H. pylori*. It would be very useful to have such a test with the same accuracy for the follow-up of this infection on animal models of gastric infection. The aim of the present study was to validate the use of UBT for detection of *H. pylori* infection in C57Bl/6 mice.

**Material and Methods:** Thirty-one female C57Bl/6 mice underwent gavage three times with either 3 × 10^9^ viable *H. pylori* (n = 16) or saline (n = 15). After 2 months, mice were fasted for 14 hours and UBT was performed using 300 µg of 13C-urea. We compared the number of THP-1 cells that expressed cathepsin X after priming with *H. pylori* antigens or after adding polymixin B, that binds to lipid A, to *H. pylori* antigens. There was a statistically significant difference in expression of cathepsin X in the group Hp158/189. We discovered that adding polymixin B to *H. pylori* antigens effects the expression of cathepsin X. Expression was higher in the group 158/189 than in the group 108/152.

The patients 108/152 did not eradicate the *H. pylori* because the antigens from these strains of *H. pylori* are less immunogenic than the antigens from strains 158/189. When we compared the level of IFN-γ, we discovered that strains 108/152 produced weaker immune response than the strains 158/189. Most probably cathepsin X plays a vital role in this process.

**Abstract no.: P04.18**

Role of 13C-Urea Breath Test in Experimental Model of Mice *H. pylori* Infection

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**Aim:** The 13C-urea breath test (UBT) is sensitive and specific for detection of human infection with *Helicobacter pylori*. It would be very useful to have such a test with the same accuracy for the follow-up of this infection on animal models of gastric infection. The aim of the present study was to validate the use of UBT for detection of *H. pylori* infection in C57Bl/6 mice.

**Material and Methods:** Thirty-one female C57Bl/6 mice underwent gavage three times with either 3 × 10^9^ viable *H. pylori* (n = 16) or saline (n = 15). After 2 months, mice were fasted for 14 hours and UBT was performed using 300 µg of 13C-urea. After UBT, mice were killed and the stomach was removed and processed for histology and polymerase chain reaction (PCR) for *H. pylori* detection.

**Results:** Using PCR as gold standard, the positivity of UBT and histology was 93.75% and 68.75%, respectively. The specificity of UBT and histology was 100% and 93.75% for PCR, respectively, and 100% and 75%, for histology, respectively. The UBT cut-off was 3.0‰, δPDB.

**Conclusions:** UBT showed to be a reliable method for the detection of *H. pylori* infection in C57Bl/6 mice, even better than histology. The use of UBT in experimental models of *H. pylori* infection is useful to detect the presence of the bacterium without the need to sacrifice the mice. This strategy would be of great value when performing *H. pylori* chronic mice infection studies.

**Abstract no.: P04.19**

A Multiepitope DNA Vaccine Based on Five Antigens of *H. pylori*

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Epitope-based vaccines present several advantages, as they potentiate the specific immunity and simultaneously avoid the side-effect of other epitopes, as for example an exacerbated inflammatory process. Furthermore, it is possible to include a wider range of target antigens covering this way the diversity of *Helicobacter pylori*. Based on immunoprotoemic data the following five antigenic targets were selected: VacA, NapA, HpA1, Omp9, and HomB. However, there is little information concerning the genetic polymorphism of these specific targets in the literature. In the present work, the target genes were amplified by polymerase chain reaction and subsequently sequenced. To each sequenced gene, the more conserved and immunogenic epitopes were selected based on the Jameson–Wolf and by both AMPHI and Rothbard–Taylor methods, respectively. The final selection of epitopes will be based on the analyses of MHC-binding prediction conducted for each human leukocyte antigen (HLA) allele, using the IMGT database, considering the HLA alleles frequency in the human population. The idea is to select the conserved and immunogenic epitopes that simultaneously will represent the antigen variability among *H. pylori*, and will be recognized by a broad range of the human population.

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**Abstract no.: P04.20**

Assessment of *H. pylori* Colonization in Guinea Pig by Immunologic Methods

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**Background:** Animal models have been useful in investigating the pathogenicity of bacteria as well as efficiency of new therapeutic compounds and vaccines. In our previous study we proposed that guinea pig could be an appropriate animal model for research on colonization of *Helicobacter pylori*. Colonization of *H. pylori* was assessed by performing polymerase chain reaction (PCR) on fresh stool of animals. In this study indirect immunofluorescence assay (IFA) and *H. pylori* stool antigen test (HpSA) were recruited for assessment of *H. pylori* colonization.

**Methods:** After PCR-confirmed *H. pylori* colonization in guinea pig, IFA was performed by anti-*H. pylori* IgG, as the first antibody and anti-Mouse IgG-FITC antibody as the second antibody. Evans blue dye was also used for creating color contrast. HpSA was performed by ELISA kit. *H. pylori* and Escherichia coli were used as positive and negative controls, respectively.

**Results:** IFA of *H. pylori* as positive control demonstrated spiral-shape bacteria with green fluorescence color. IFA demonstrated...
presence of *H. pylori* crude antigen in the stool sample of infected guinea pigs. *E.coli* as negative control stained red by Evans blue dye. Occurrence of *H. pylori* crude antigen was also confirmed by ELISA.

**Discussion:** In recent years Mongolian gerbil has been introduced as an appropriate model for research on *H. pylori* colonization. Positive results of PCR, IFA, and stool antigen tests indicate that guinea pig could be a useful substitute for Mongolian gerbil. This study is ongoing and the methods mentioned above are going to be recruited for assessment of the efficiency of new synthetic compounds in treatment of *H. pylori*-infected guinea pigs.

## P05 Other Helicobacters, Hepatobiliary Diseases, Esophageal, and Extradigestive Diseases

**Abstract no.: P05.01**

**Dietary Factors and *H. pylori* Infection**

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*Helicobacter pylori* infection is very common in Poland, which is found in 85–95% of adult population. It may be at least partly related to improper lifestyle, especially diet.

The aim of the study was to examine if some dietary factors contribute to *H. pylori* infection. Studied patients were referred for endoscopic examination of the upper digestive tract in 2002–2007 to explain the cause of dyspeptic disorders. In some patients *H. pylori* infection was diagnosed for the first time, in others reinfection occurred after successful treatment in the past. Patients who have not been infected or reinfected were included into the control group. The respondents were interviewed retrospectively on their dietary habits.

A lower frequency of fermented dairy products, vegetables, and fruit consumption was noted among persons with *H. pylori* infection as compared to the control group. In the examined group 43–47% declared to eat fermented dairy products frequently (at least five times a week) while in the control group 95–96%; in the case of vegetables consumption these percentages were 74% and 77–87% and in the case of fruit consumption 51–58% and 70–76%, respectively.

Obtained results indicate that high consumption of fermented dairy products containing probiotic bacteria, mainly *Lactobacillus*, and vegetables and fruit – source of antioxidants such as vitamin C, may decrease the risk of *H. pylori* infection.

**Abstract no.: P05.02**

**Helicobacter felis is the Major Gastric Helicobacter Species in Dogs**

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**Background:** *Helicobacter* is frequently demonstrated in gastric biopsy specimens obtained from dogs; however, their role as potential pathogen in gastritis has not been clearly established.

**Aim:** To detect *Helicobacter* species in the gastric mucosa of dogs and to correlate the presence of *Helicobacter* infection with gastritis.

**Animals:** Biopsy samples were taken during gastric endoscopy from the fundus of 20 dogs with or without signs of gastrointestinal disease.

**Methods:** Histopathologic techniques, Helicobacteraceae family- and *Helicobacter* genus-specific polymerase chain reaction (PCR) assays, and fluorescence in situ hybridization (FISH) analysis were used to detect *Helicobacter* spp. The identity of the species was obtained by amplifying a 764-bp 16S rRNA gene sequence specific to Helicobacteraceae.

**Results:** Nineteen dogs showed mild to severe gastritis in the fundus, and only one had a healthy gastric mucosa. *Helicobacter* spp. DNA was detected in 18 of 19 dogs with gastritis and only one with a normal gastric mucosa. The sequences of DNA amplicons (600–711 bp) shared 99–100% identity with the 16S rRNA genes of *H. felis*, *H. salmonis*, and *Helicobacter* sp. in 79%, 10.5%, and 10.5% of the dogs, respectively. Using FISH, the presence of *Helicobacter* species was evidenced in 19 animals (100% coincidence).

**Conclusions:** Venezuelan pet dogs are frequently colonized by *H. felis* without a significant correlation between infection and degree of gastritis, suggesting the possibility that dogs may act as source of non-*H. pylori* *Helicobacter* spp. infection for humans.

**Abstract no.: P05.03**

**Helicobacter spp. DNA in Mucosa of Swedish Patients with Cholecystitis**

P. H. Karagin, U. Stenram, H. Nilsson, T. Wadström and A. Ljungh

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**Background:** *Helicobacter* DNA in cholecystitis mucosa has been reported with prevalence from 2% to 83% in different countries. Several authors claim that *Helicobacter* is especially found in areas of gastric metaplasia. We examined a series of patients from Southern Sweden.

**Methods:** Paraffin-embedded samples (5 mg tissue) from the mucosa of 55 patients with cholecystitis were studied by *Helicobacter* DNA-specific PCR assay and sequence analysis.

**Results:** *Helicobacter* DNA was found in nine of 36 so far examined patients. Sequence analysis displayed close similarity with *H. pullorum* in all cases and lower similarity with *H. canadensis*. There was very little gastric metaplasia.
presence of \textit{H. pylori} crude antigen in the stool sample of infected guinea pigs. 	extit{E.coli} as negative control stained red by Evans blue dye. Occurrence of \textit{H. pylori} crude antigen was also confirmed by ELISA.

\textbf{Discussion:} In recent years Mongolian gerbil has been introduced as an appropriate model for research on \textit{H. pylori} colonization. Positive results of PCR, IFA, and stool antigen tests indicate that guinea pig could be a useful substitute for Mongolian gerbil. This study is ongoing and the methods mentioned above are going to be recruited for assessment of the efficiency of new synthetic compounds in treatment of \textit{H. pylori}-infected guinea pigs.

\section*{P05 Other Helicobacters, Hepatobiliary Diseases, Esophageal, and Extradigestive Diseases}

\subsection*{Abstract no.: P05.01 Dietary Factors and \textit{H. pylori} Infection}

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\textit{Helicobacter pylori} infection is very common in Poland, which is found is 85–93\% of adult population. It may be at least partly related to improper lifestyle, especially diet. The aim of the study was to examine if some dietary factors contribute to \textit{H. pylori} infection.

Studied patients were referred for endoscopic examination of the upper digestive tract in 2002–2007 to explain the cause of dyspeptic disorders. In some patients \textit{H. pylori} infection was diagnosed for the first time, in others reinfection occurred after successful treatment in the past. Patients who have not been infected or reinfected were included into the control group. The respondents were interviewed retrospectively on their dietary habits.

A lower frequency of fermented dairy products, vegetables, and fruit consumption was noted among persons with \textit{H. pylori} infection as compared to the control group. In the examined group 43–47\% declared to eat fermented dairy products frequently (at least five times a week) while in the control group 95–96\%; in the case of vegetables consumption these percentages were 74\% and 77–87\% and in the case of fruit consumption 51–58\% and 70–76\%, respectively.

Obtained results indicate that high consumption of fermented dairy products containing probiotic bacteria, mainly \textit{Lactobacillus}, and vegetables and fruit – source of antioxidants such as vitamin C, may decrease the risk of \textit{H. pylori} infection.

\subsection*{Abstract no.: P05.01 Helicobacter pylori Infection}

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\textbf{Background:} \textit{Helicobacter} is frequently demonstrated in gastric biopsy specimens obtained from dogs; however, their role as potential pathogen in gastritis has not been clearly established.

\textbf{Aim:} To detect \textit{Helicobacter} species in the gastric mucosa of dogs and to correlate the presence of \textit{Helicobacter} infection with gastritis.

\textbf{Animals:} Biopsy samples were taken during gastric endoscopy from the fundus of 20 dogs with or without signs of gastrointestinal disease.

\textbf{Methods:} Histopathologic techniques, \textit{Helicobacteraceae} family- and \textit{Helicobacter} genus-specific polymerase chain reaction (PCR) assays, and fluorescence in situ hybridization (FISH) analysis were used to detect \textit{Helicobacter} spp. The identity of the species was obtained by amplifying a 764-bp 16S rRNA gene sequence specific to \textit{Helicobacteraceae}.

\textbf{Results:} Nineteen dogs showed mild to severe gastritis in the fundus, and only one had a healthy gastric mucosa. \textit{Helicobacter} spp. DNA was detected in 18 of 19 dogs with gastritis and only one with a normal gastric mucosa. The sequences of DNA amplicors (600–711 bp) shared 99–100\% identity with the 16S RNA genes of \textit{H. felis}, \textit{H. salomonis}, and \textit{Helicobacter} sp. in 79\%, 10.5\%, and 10.5\% of the dogs, respectively. Using FISH, the presence of \textit{Helicobacter} species was evidenced in 19 animals (100\% coincidence).

\textbf{Conclusions:} Venezuelan pet dogs are frequently colonized by \textit{H. felis} without a significant correlation between infection and degree of gastritis, suggesting the possibility that dogs may act as source of non-\textit{H. pylori} \textit{Helicobacter} spp. infection for humans.

\subsection*{Abstract no.: P05.03 \textit{Helicobacter} spp. DNA in Mucosa of Swedish Patients with Cholecystitis}

\textbf{P. H. Karagin, U. Stenram, H. Nilsson, T. Wadström and A. Ljungh}\n‘Medical Microbiology and Immunology, Lund, Sweden; ‘Pathology and Bacteriology, Lund, Sweden

\textbf{Background:} \textit{Helicobacter} DNA in cholecystitis mucosa has been reported with prevalence from 2\% to 83\% in different countries. Several authors claim that \textit{Helicobacter} is especially found in areas of gastric metaplasia. We examined a series of patients from Southern Sweden.

\textbf{Methods:} Paraffin-embedded samples (5 mg tissue) from the mucosa of 55 patients with cholecystitis were studied by \textit{Helicobacter} DNA-specific PCR assay and sequence analysis.

\textbf{Results:} \textit{Helicobacter} DNA was found in nine of 36 so far examined patients. Sequence analysis displayed close similarity with \textit{H. pullorum} in all cases and lower similarity with \textit{H. canadensis}. There was very little gastric metaplasia.
Discussion: The prevalence was higher than in a recent German study but lower than in studies from a few non-Western countries. H. pylori has been found by one previous author, H. canadensis by none. More cases are being examined. The paraffin-embedded specimens are composed of epithelial as well as stromal elements, and Helicobacter may be present only in the epithelium. The presence of non-H. pylori species may not be dependent on gastric metaplasia.

Conclusion: Helicobacter DNA in a Swedish patient material of cholecystitis mucosa was more prevalent than in Germany but higher in patients with alcohol abuse than in control group. This patients than in control group. The eradication rate was significantly decreased with time of duration of alcohol abuse.

Abstract no.: P05.04
Eradication of H. pylori Infection in Patients with Alcohol-Induced Pancreatitis

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Aim: The aim of this study was to evaluate efficacy of Helicobacter pylori eradication in patients with alcohol-induced pancreatitis and compared with nonalcoholic patients.

Methods: The patients with acute pancreatitis were divided into two groups. Group A: 35 patients (26 male/9 female), mean age 44 years, with alcohol-induced pancreatitis, and group B: 35 patients (20 male/15 female), mean age 41 years, with nonalcoholic-induced pancreatitis. All patients had upper gastrointestinal endoscopy. H. pylori infection was confirmed by gastric histology. The patient from group A drank four to eight glasses of wine or 6–8.5 L of beer per week. A triple therapy with amoxicillin (1 g two times a day), claritromycin (500 mg two times a day) as first cycle, or amoxicillin (1 g two times a day), claritromycin (500 mg two times a day), and lansoprazole (30 mg two times a day) as second cycle, was given to both groups for 10 days. The cure was defined as the absence of H. pylori infection 6 weeks after therapy.

Results: A higher H. pylori infection was found in group B, in nonalcoholic patients 68% versus 42% in alcoholic patients (p > .001). In: alcoholic patients in group A eradication of H. pylori infection was 82% versus 60% in nonalcoholic patients (p > .001). Higher wine and beer consumption were associated with an additional reduction in the risk of infection. The rate of infection decreased with time of duration of alcohol abuse.

Conclusion: H. pylori infection was lower in group of alcoholic patients than in control group. The eradication rate was significantly higher in patients with alcohol abuse than in control group. This study suggests that moderate alcohol consumption may facilitate spontaneous elimination of H. pylori infection among adults.

Abstract no.: P05.05
Presence of H. pylori Infection in Cirrhosis Patients is Related to Slower Recovery from Hepatic Encephalopathy

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Background/Aims: Hepatic encephalopathy is a frequent complication of liver cirrhosis. It has been suggested that Helicobacter pylori contributes to hyperammonemia in cirrhosis. We aimed to investigate the relationship between H. pylori infection, blood ammonia concentration, and hepatic encephalopathy in cirrhosis patients.

Methods: Thirty-nine patients treated in the intensive care unit in clinical center of Serbia with decompensated liver cirrhosis entered the study (35 males, mean age 58 ± 8). They were divided into two groups based on the presence of H. pylori infection. Twenty-six patients were H. pylori positive and 13 H. pylori negative. Patients were evaluated for demographic and clinical data, liver impairment, blood ammonia concentration, and HE.

Results: HE lasted longer in H. pylori-positive patients (median 6 days, range 1–20 days) than in H. pylori-negative patients (median 3 days, range 1–11 days) p < .05. Higher levels of blood ammonia were also found in H. pylori-positive patients (H. pylori positive 63.5 ± 48, H. pylori negative 43 ± 27 µmol/L, p > .05) but difference was not statistically significant. No difference between groups was observed in clinical and demographic data, etiology of cirrhosis, presence of ascites, kidney function impairment, presence of gastrointestinal bleeding, or final outcome.

Conclusion: Presence of H. pylori infection is related to longer duration of HE, thus H. pylori eradication may be helpful for treatment and prevention of HE.

Abstract no.: P05.06
Detection of H. pylori in the Hepatobiliary System of Patients with Biliary Tract Diseases

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Aim: Despite several recent reports on the detection of Helicobacter pylori DNA in human bile, there are still uncertainties concerning the correlation of these findings with biliary tract and liver diseases.

Material and Methods: Using polymerase chain reaction (PCR), we detected the presence of H. pylori in bile samples, gallbladder, and liver biopsy specimens from 102 persons, of which 72 adults with hepatobiliary diseases (HBD) and liver cirrhosis. Of the 102 patients, representing the control group, 30 were without biliary diseases. Bile samples were obtained by duodenal intubations; gallbladder mucus samples were obtained by resection of the gallbladder and liver. Among the 72 patients, 42 had noncalculous cholecystitis, 19 had calculous cholecystitis and 11 with liver cirrhosis. The detection of H. pylori DNA was performed by polymerase chain reaction using H. pylori-specific primers for ureC according to the manufacturer's recommendation (Lytech, Russia).
Results: *H. pylori* was found in 31 samples (liver, gallbladder, bile duct, and bile) of patients with liver cirrhosis and chronic cholecystitis. In accordance with different hepatobiliary pathology, 21 (50.0%) samples of 42 with noncalculous cholecystitis, 8 (72.7%) samples of 11 with liver cirrhosis, and only in 2 (10.5%) of the 19 samples with calculous cholecystitis were positive. In the control group none of the samples showed the presence of *H. pylori*.

Conclusion: The presence of *H. pylori* in hepatobiliary system may tell about the influence of the bacteria in the development of hepatobiliary diseases.

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**Abstract no.: P05.07**

**The Clinical Result of *H. pylori* Eradication in Patients with *H. pylori*-positive Idiopathic Thrombocytopenic Purpura of St. Mary Hospital**

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Background: *Helicobacter pylori* is the cause of gastric and multiple extragastric diseases. A beneficial effect of *H. pylori* eradication in patients with *H. pylori*-positive idiopathic thrombocytopenic purpura (ITP) has been reported by several investigators; however, its efficacy varies between countries. The response rate of *H. pylori* eradication in *H. pylori*-positive chronic and acute ITP patients at St. Mary Hospital was investigated

Method: Between September 2005 and April 2007, a total of 18 patients diagnosed with ITP were included in the study. The *H. pylori* infection was assessed by urea breath test. *H. pylori* eradication was performed on *H. pylori*-positive ITP patients with the amoxicillin, clarithromycin, and proton pump inhibitor regimen for 7 days. We investigated the efficacy of *H. pylori* eradication on platelet recovery in patients with *H. pylori*-positive ITP.

Result: Eighteen patients with ITP were evaluated, including 8 males with 10 females. Mean age of patients was 44.9 ± 17 years and 12 (63.2%) were positive for *H. pylori*. Six are outpatients and others are in admission state. Eradication was performed and three (25%) had a significant increase in platelet counts after treatment and all of them were located in group with successful *H. pylori* eradication. Platelet count of responders was increased from 10,000 ± 8660/µL to 111,694 ± 40,628/µL, and mean follow-up time was 24.3 ± 2.5 months.

Discussion: Our analysis shows the efficacy of *H. pylori* eradication in patients with *H. pylori*-positive ITP at St. Mary Hospital. There was no significant relationship between platelet response and clinical characteristics of *H. pylori*-positive patients.

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**Abstract no.: P05.08**

**H. pylori and Iron-Deficiency Anemia: A Meta-analysis of Case–Control Studies**

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Background: Recently, there has been a growing body of evidence suggesting a relationship between *Helicobacter pylori* gastritis and hypol ferritinemia or iron-deficiency anemia (IDA).

Aim: To systematically review the role of *H. pylori* infection in hypol ferritinemia and IDA, and to perform a meta-analysis of case–control studies.

Methods: Selection of studies: Case–control studies comparing (a) the prevalence of *H. pylori* infection in patients with and without IDA/hypoferritinemia, and (b) the prevalence of IDA/hypoferritinemia in patients with and without *H. pylori* infection. Search strategy: electronic and manual bibliographical searches. Data synthesis: Meta-analysis combining the odds ratios (OR).

Results: (a) Eight studies compared the prevalence of *H. pylori* infection in patients with (637 patients) and without (2305 patients) IDA, showing a higher prevalence of *H. pylori* infection in anemic patients [35% vs 22%; OR = 1.7; 95% confidence interval (CI) = 1.1–2.5]. Only one study compared the prevalence of infection in patients with and without hypoferritinemia (29% vs 19%; p < .05). (b) Seven studies compared the prevalence of IDA in patients with (1190 patients) and without (678 patients) *H. pylori* infection, showing a higher prevalence of IDA in *H. pylori*-positive patients (17% vs 14%; OR = 2.3; 95% CI = 1.2–4.3). Finally, five studies compared the prevalence of hypoferritinemia in patients with (297 patients) and without (129 patients) *H. pylori* infection, showing a higher prevalence of hypoferritinemia in *H. pylori*-positive patients (33% vs 13%; OR = 1.7; 95% CI = 1.1–2.7). Results were heterogeneous for all comparisons.

Conclusion: Epidemiologic studies suggest an association between *H. pylori* infection and lower iron stores or IDA. However, these data should be interpreted with caution due to marked heterogeneity among studies.

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**Abstract no.: P05.09**

**Effect of *H. pylori* Eradication on Iron-Deficiency Anemia: A Meta-analysis of Randomized Clinical Trials**

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Background: Reversal of iron-deficiency anemia (IDA) after successful cure of *Helicobacter pylori* infection has recently been observed in several randomized clinical trials.

Aim: To systematically review the effect of *H. pylori* eradication on hypoferritinemia and IDA, and to perform a meta-analysis of randomized clinical trials comparing *H. pylori* eradication treatment and iron administration.


Results: Five studies compared the increase in hemoglobin levels achieved with *H. pylori* eradication (plus iron) treatment and with iron administration alone in patients with IDA, showing a higher efficacy in the eradication group [SMD = 2.9; 95% confidence interval (CI) = 0.5–5.3]; however, two studies could not demonstrate the beneficial effect of antibiotic treatment. On the other hand, four studies compared the increase in serum ferritin concentrations after *H. pylori* eradication treatment and after iron administration in patients with IDA, showing, again, a higher efficacy in the group receiving eradication therapy.
(SMD = 6.6; 95% CI = 2.7–10.4). Results were markedly heterogeneous for all comparisons.

**Conclusion:** Some studies have demonstrated reversal of IDA after successful cure of *H. pylori* infection, and the meta-analysis of randomized controlled trials showed that *H. pylori* eradication (plus iron) is more effective than iron administration alone for the treatment of lower iron stores or IDA. However, these data should be interpreted with extreme caution due to marked heterogeneity among studies.

### Abstract no.: P05.10

**Culture of H. pylori from Heterotropic Gastric Mucosa**

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In April 2008 a 61-year-old male patient presented with tickle of the throat, globus pharyngeus, and heartburn. Endoscopy of the esophagus and stomach was performed, where subpharyngeal localized heterotropic gastric mucosa (HGM) and a chronic gastritis were diagnosed. The histologic examination was suspicious for *H. pylori* infection of the stomach and the HGM. After consulting the NRC, we tried to culture *H. pylori* from biopsies of the stomach and the HGM. *H. pylori*, susceptible to clarithromycin, levofloxacin, tetracycline, rifampicin, metronidazole, and amoxicillin with identical minimum inhibitory concentrations, was cultured from both sites. Molecular typing revealed a cagA-positive strain with a vacA S1a/m1 genotype. The patient was treated with a French triple therapy (proton pump inhibitor + amoxicillin + clarithromycin) for 10 days. All performed tests for eradication control (endoscopy with samples for rapid urease test, histology, culture and real-time PCR and stool antigen ELISA) were negative. Furthermore, the patient reported of clinical improvement. In conclusion, *H. pylori* colonization of patches of heterotropic gastric mucosa has been described in the literature. For diagnosis histologic examination is used. To our knowledge this is the first case report of positive *H. pylori* culture from HGM.

### Abstract no.: P05.11

**Carditis can Partially Regress After H. pylori Eradication but not Proton Pump Inhibitor Treatment**


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**Aim:** To define the effect of *Helicobacter pylori* eradication and antireflux treatment in carditis and intestinal metaplasia of the gastric cardia.

**Patients-methods:** Two hundred and forty patients with gastroesophageal reflux disease (GERD) (mean age 59 ± 15 years, 145 male) and 240 controls without GERD (mean age 58 ± 17 years, 138 male) after gastroscopy with biopsies were started on omeprazole 20 mg twice daily for 1 year plus a 10-day *H. pylori* eradication regimen if *H. pylori* positive. Finishing treatment, we repeated endoscopy with biopsies, on omeprazole and performed 13C-urea breath test, off omeprazole if *H. pylori* positive. The Sydney classification was used for carditis/intestinal metaplasia.

**Results:** Cardiac mucosa was identified in 180 (75%) controls, 220 (92%) refluxers (p < .001); carditis in 102 (43%) controls, 152 (63%) refluxers (p < .001) *H. pylori* positive: 50 (21%) controls, 76 (32%) refluxers (p = .007); *H. pylori* negative: 52 (22%) controls, 76 (32%) refluxers (p = .011). Intestinal metaplasia of the cardia was found in 45 (19%) controls, 84 (35%) refluxers (p < .001). Forty-one controls with carditis and 61 refluxers successfully eradicated *H. pylori*. Of them 29 controls, 31 refluxers present no carditis in the follow-up endoscopy. After 1 year, 73 (30%) controls, 123 (51%) refluxers presented carditis (p < .001). During follow up there was no change in the severity of intestinal metaplasia, carditis regressed in 71% (n = 29) of *H. pylori*-positive controls who eradicated *H. pylori*, 51% (n = 31) refluxers; it increased by 0.5 ± 0.1 grades in controls, 0.3 ± 0.1 in refluxers who persisted *H. pylori*, while remained rather unchanged in *H. pylori*-negative patients and controls. Five refluxers developed dysplasia.

**Conclusions:** 1. Both carditis and intestinal metaplasia of the gastric cardia are more frequent in GERD patients. 2. Carditis but not intestinal metaplasia can regress less frequently in *H. pylori*-positive patients than controls after *H. pylori* eradication. 3. High dose omeprazole treatment has little effect on carditis.

### Abstract no.: P05.12

**H. pylori Colonization of the Adenotonsillar Tissue: Fact or Fiction?**


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**Aim:** To define the effect of *Helicobacter pylori* eradication and antireflux treatment in carditis and intestinal metaplasia of the gastric cardia.

Gastric infection with *Helicobacter pylori* is the most common chronic infection worldwide. One of the routes of transmission of the infection is the oral route. Molecular techniques have allowed the detection of *H. pylori* DNA in samples of the oral cavity, although culture of *H. pylori* from these types of samples has been sporadic. Studies have tried to demonstrate the presence of *H. pylori* in adenotonsillar tissue, with contradictory results. Our aim was to clarify whether the adenotonsillar tissue may constitute an extragastric reservoir for *H. pylori*.
Sixty-two patients proposed for adenoidectomy or tonsillectomy were enrolled. A total of 101 samples, 55 adenoid and 46 tonsils, were obtained. Patients were characterized for the presence of anti-H. pylori antibodies by serology. On each surgical sample rapid urease test, immunohistochemistry. PCR-DEIA directed to the vacA gene of H. pylori, and FISH with a peptide nucleic acid probe for H. pylori were performed.

In the study population, 33% of the individuals had anti-H. pylori antibodies. Rapid urease test was positive on samples of three patients, all with positive serology. Immunohistochemistry was positive on two patients, all with negative serology. All positive cases by rapid urease test or immunohistochemistry were negative by FISH. PCR-DEIA directly in adenotonsillar tissue was negative in all samples.

In conclusion, the adenotonsillar tissue does not constitute an extragastric permanent reservoir for H. pylori infection, at least in this population from the North of Portugal.

Abstract no.: P05.13
Does Eradication of H. pylori Infection Delay the Development Lung Cancer?

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Background: In several studies Helicobacter pylori infection has been associated with lung cancer. Nearly all H. pylori-infected subjects have permanently elevated levels of circulating antibodies. Rapidly falling antibody titers after antimicrobial medications indicate cure of infection.

Methods: Altogether 26,705 consecutive patients tested in 1986–1998 for H. pylori antibodies were allocated to three subcohorts: 1, seropositive patients without confirmation of cure (Hp+NOCURE); 3, seropositive patients with rapidly falling antibody titers (Hp+ERADICATED); and 3, seronegative patients (Hp−). Subsequent lung cancers were identified from the Finnish Cancer Registry until the end of 2006. The risk ratios (RRs) with 95% confidence intervals (95%CI) were defined in a Poisson regression analysis using the Hp+NOCURE as the reference.

Results: Among 11,633 Hp+NOCURE, 3650 Hp+ERADICATED, and 11,422 Hp− patients, followed for a mean of 9.3, 10.4, and 10.9 years, subsequent lung cancers were found in 161, 33, and 11,422 Hp− patients, respectively. For the Hp+ERADICATED, the RR was 0.49 (95%CI 0.27–0.89) for the first five follow-up years but increased from the sixth follow-up year to 0.96 (95% CI 0.65–1.42). The RR for Hp− were 0.63 (95% CI 0.40–0.99) and 0.50 (95%CI 0.33–0.77) for the same periods, respectively.

Conclusions: Cured H. pylori infection led to a significantly decreased incidence of lung cancers for the first five follow-up years. During the whole follow up, significantly fewer lung cancers were found in the Hp− cohort than in the Hp+NOCURE cohort.

Abstract no.: P05.14
Reflux Esophagitis in Children: Does Endoscopy Predict the Disease?

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The concordance between gastroesophageal reflux (GER) symptoms and endoscopic and histopathologic findings is still obscure in children. The aim was to evaluate the correlation between symptoms of GERD and endoscopic and histopathologic esophagitis in children who underwent endoscopy and to define the role of H. pylori gastritis in the reflux esophagitis as well.

A total of 59 subjects who had complaints suggesting GERD underwent endoscopy. Reflux symptoms were evaluated by using either Orenstein or Manterola scoring systems depending on the age of patients. The endoscopic and histopathologic diagnosis and severity of reflux esophagitis were assessed by Savary–Miller and Vandenplas grading systems, respectively. The updated Sydney score was used to assess H. pylori gastritis.

Mean age of the study group was 8.9 ± 4.4 years, and 31 (52.5%) had GERD according to the symptom scores. Thirty-one (52.5%) children had endoscopic and 47 (79.7%) patients had histopathologic esophagitis. The correlation between symptom score and endoscopic findings was not significant. However, the correlation between symptom score and histopathologic esophagitis was significant (p < .01). Eighteen of 28 (64.3%) endoscopically normal patients had histopathologic esophagitis. H. pylori was positive in 74.6% of the patients, and 29 of 44 H. pylori-infected children had gastritis. There was no correlation between H. pylori gastritis and histologic esophagitis in our group.

The clinical findings are very important for the suspicion of GER in childhood. Since endoscopic findings are not very leading in children, esophageal biopsies should be obtained in every child during endoscopy. H. pylori gastritis is not correlated with reflux esophagitis in this study.

Abstract no.: P05.15
The Expressed Interrelation of Heartburn with Esophagitis, Barrett Esophagus, and H. pylori-Associated Peptic Ulcer in Patients with Various Age–Sex Characteristics Among Inhabitants of Eastern Siberia

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Aim: To study interrelation of heartburn, esophagitis, and Helicobacter pylori-associated peptic ulcer in representatives of various age–sex groups.

Methods: We carried out cross-section epidemiologic study using the modified questionnaire of Mejo Clinic (Locke G.R., Talley N.J. et al., 1994). Prevalence of heartburn was examined in 506 men
in a group of this association, we compared vascular and inflammatory factors, *Helicobacter pylori*. Recent case–control studies reported an association between *H. pylori* infection and Alzheimer dementia (AD). To explore this association, we compared vascular and inflammatory factors, in a group of *H. pylori*-infected and noninfected AD patients. We studied serum and cerebrospinal fluid (CSF) samples from a group of AD patients. We assessed: 1, vascular comorbidities and cognitive status; 2, C-reactive protein level (CRP), homocysteine level, and cytokines [interleukin (IL)-1 beta, IL-6, IL-8, tumor necrosis factor (TNF)-alpha] for serum; 3, cytokines, phospho-Tau protein (pTau), and amyloid beta peptide levels from LCR; and 4, brain magnetic resonance imaging data (Fazekas scale). *H. pylori* infection was defined by a positive ELISA and/or immunoblot test (CagA). A total of 53 patients were included (23 men and 30 women, mean age: 68.5 ± 8.7 years). *H. pylori* infection was diagnosed in 37 (69%) patients, including 32 (86.5%) CagA+. *H. pylori* infection was associated with a decreased pepsinogen ratio (p = .008) and was significantly associated with vascular factors, i.e., positive correlation with homocysteinemia (r = 0.44, p = .001) and increased MRI white matter hyper-intensities (p = .04). *H. pylori* infection was also associated with chronic inflammation, i.e. positive correlation with CRP (r = 0.31, p = .03) and fibrinogen (r = 0.34, p = .02) levels and increased CSF TNF-alpha (p = .02) and IL-8 (p = .004) levels. *H. pylori* infection was statistically associated with the presence of neurodegeneration markers, i.e. increased pTau (p = .01). *H. pylori* infection is associated with AD by two mechanisms which are probably linked: systemic and CSF inflammation and increasing brain vascular lesions. Chronic infection might be correlated to neurodegeneration lesions via both of these factors.

**Abstract no.: P05.16**

*H. pylori* Chronic Infection and Alzheimer Disease: Vascular or and Inflammatory Association?

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Recent case–control studies reported an association between *Helicobacter pylori* infection and Alzheimer dementia (AD). To explore this association, we compared vascular and inflammatory factors, in a group of *H. pylori*-infected and noninfected AD patients. We studied serum and cerebrospinal fluid (CSF) samples from a group of AD patients. We assessed: 1, vascular comorbidities and cognitive status; 2, C-reactive protein level (CRP), homocysteine level, and cytokines [interleukin (IL)-1 beta, IL-6, IL-8, tumor necrosis factor (TNF)-alpha] for serum; 3, cytokines, phospho-Tau protein (pTau), and amyloid beta peptide levels from LCR; and 4, brain magnetic resonance imaging data (Fazekas scale). *H. pylori* infection was defined by a positive ELISA and/or immunoblot test (CagA). A total of 53 patients were included (23 men and 30 women, mean age: 68.5 ± 8.7 years). *H. pylori* infection was diagnosed in 37 (69%) patients, including 32 (86.5%) CagA+. *H. pylori* infection was associated with a decreased pepsinogen ratio (p = .008) and was significantly associated with vascular factors, i.e., positive correlation with homocysteinemia (r = 0.44, p = .001) and increased MRI white matter hyper-intensities (p = .04). *H. pylori* infection was also associated with chronic inflammation, i.e. positive correlation with CRP (r = 0.31, p = .03) and fibrinogen (r = 0.34, p = .02) levels and increased CSF TNF-alpha (p = .02) and IL-8 (p = .004) levels. *H. pylori* infection was statistically associated with the presence of neurodegeneration markers, i.e. increased pTau (p = .01). *H. pylori* infection is associated with AD by two mechanisms which are probably linked: systemic and CSF inflammation and increasing brain vascular lesions. Chronic infection might be correlated to neurodegeneration lesions via both of these factors.

**Abstract no.: P05.17**

Symptoms of Gastroesophageal Reflux in the Population: Association with Serum Biomarkers

O. V. Reshetnikov,* S. A. Kurilovich,* S. A. Krotova,† V. A. Krotova* and E. D. Pylenkova*†

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Gastroesophageal reflux (GER) was associated with serum pepsinogen level in some studies; however, its interrelationship with *Helicobacter pylori* infection is still a matter of controversy.

**Material and Methods:** Two hundred and sixty-four subjects randomly selected from the general population were studied in Novosibirsk, Western Siberia (127 males, 137 females aged 45–69 years, mean age 59.4 years). Bowel Disease Questionnaire was used to study GER symptoms. Serum biomarkers (*H. pylori* antibodies, pepsinogen I, and gastrin-17) were assessed with Gastropanel (Biokit, Finland). Anti-*H. pylori* IgG CagA antibodies were evaluated using domestic ELISA kits (Joint-Stock Company Vector-Best, Novosibirsk, Russia).

**Results:** GER symptoms (at least once a month) were reported by 27.3% not depending on sex and age. In univariate analysis, presence of GER symptoms was associated with elevated pepsinogen I concentration (mean 123.2 vs 94.1 ng/mL, p < .0001), low gastrin-17 concentration (median 4.48 vs 6.34 pmol/L, p = .014), and CagA negativity (76.1 vs 60.9%, p = .016), but not with *H. pylori* status.

Multivariate analysis showed independent association of GER with pepsinogen I concentration > 100 ng/mL (OR = 3.0, 95% CI 1.6–5.5, p = .0004), and CagA negativity (OR = 2.3, 95% CI 1.2–4.3, p = .01).

**Conclusion:** Serologic evidence of nonatrophic, non-CagA-infected gastric mucosa is associated with gastroesophageal reflux symptoms in a population sample.

**Abstract no.: P05.18**

Is There Any Role of *H. pylori* in the Pathogenesis of Bronchiectasis?

T. Aydin Teke,* Y. Akyon,* E. Yalcin,* H. Ozen,* N. Cobanoglu,* S. Pekcan,* M. Kose,* D. Dogru,* N. Kiper* and U. Ozcelik*

*Hacettepe University, Ankara, Turkey; †Meram University, Konya, Turkey; ‡Erciyes University, Kayseri, Turkey*

The aim of this study was to find out whether or not *Helicobacter pylori* is responsible for lung injury in pediatric patients with bronchiectasis. The study group consisted of 29 patients with noncystic fibrosis bronchiectasis. The control group was consisted of nine individuals who did not have any documented evidence of pulmonary infection or bronchiectasis. Bronchoalveolar lavage (BAL) and fasting gastric aspirate samples (FGAS) were used to detect *H. pylori* by culture and polymerase chain reaction (PCR). Urea breath test (UBT) was performed. Gastroesophageal reflux (GER) was evaluated in *H. pylori*-positive patients. BAL culture and/or PCR was positive in 11 of 29 cases of study group. BAL culture was negative in all individuals in the control group, but PCR was positive in two of nine individuals. The FGAS cultures and/or PCR analysis were positive in 12 patients in the...
study group, while four cases were positive in the control group. Six of 11 patients were positive for H. pylori both in BAL and in FGAS. GER was detected in two of these patients. In the control group one of the two individuals was positive for H. pylori both in BAL and in FGAS, but none of these individuals had GER. UBT was positive in eight of 17 study group patients with H. pylori positivity while it was positive in one of five patients with H. pylori positivity in the control group.

According to our findings, we could suggest that H. pylori could have a role in the development and/or progression of bronchiectasis and that GER might mediate in the transfer of H. pylori to the lungs.

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Abstract no.: P05.19

Occurrence of H. pylori Infection in the Esophageal Mucosa of Symptomatic Subjects

M. Contreras, V. Salazar, M. A. Garcia-Amado, N. Reyes, M. Aparcero, O. Silva, D. Castro, R. Romero, P. Gueneau and F. Michelangeli

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Over the past few years, interest in Helicobacter pylori has extended from its role in the etiology of diseases of the stomach and duodenum to its possible role in the etiology of diseases of the esophagus. While in developing countries, the prevalence of H. pylori gastric colonization is known to be high (46 to 95%) in symptomatic subjects, there are few reports of the presence of H. pylori in the esophageal mucosa. The aim of this study was to assess the H. pylori infection in the esophagus of 85 Venezuelan symptomatic patients. Infection was detected through polymerase chain reaction (PCR) analysis of DNA extracted from esophageal and gastric biopsies, using genus- and species-specific primers. Infection in the esophagus was found by PCR in 52 patients (61%), of which 50 (59%) were cagA(+) while infection in the stomach was detected in 42 patients (49%) of which 38 (44%) were cagA(+). H. pylori infection in the esophagus was confirmed in 65 patients (76%) by FISH to visualize the bacteria within intact tissue samples. The presence of H. pylori, independently of method employed, was identified in 72 patients (85%) of which 45 (53%) have coincident positive tests, while 27 patients (32%) have positive but not coincident tests. According to our results, the prevalence of infection by H. pylori found in the esophageal mucosa is higher than that in the gastric mucosa, suggesting that H. pylori colonizes the esophagus when the esophageal mucosa is substituted by columnar epithelium.

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Abstract no.: P05.20

Risk Factors Associated with Rosacea

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Introduction: Although rosacea is a very common disease, the cause of disease is still a mystery – Helicobacter pylori infection, genetic predisposition, climatic factors, and detrimental habits are implicated as triggers of rosacea. The aim of current study is to evaluate several suspected risk factors coincidently.

Methods: Patients with rosacea from a dermatology clinic and skin-healthy controls from a randomly selected employees population enrolled the study. Skin status was evaluated by one and same dermatologist. Participants were queried for age, gender, sun-reactive skin type, and detrimental habits using a questionnaire; blood samples for detecting H. pylori serostatus were collected.

Results: Totally 145 skin-healthy controls and 172 subjects either with flushing episodes or established rosacea included the study. In multivariate analysis, rosacea patients had significantly higher chance to have photosensitive skin types [odds ratio (OR) 1.75; 95% confidence interval (CI) 1.01–3.04; p = .007], positive family history to rosacea (OR 4.31; 95% CI 2.34–7.92; p < .0001), or previous smoking status (OR 2.01; 95% CI 1.07–3.80; p = .031) compared with skin-healthy controls. There were no statistically significant differences either in gender, H. pylori serostatus, caffeine intake, alcohol consumption, occupational environment, or education level between rosacea patients and controls.

Conclusion: Rosacea is foremost associated with familial predisposition. There is no association between H. pylori infection and rosacea in current study.

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Abstract no.: P05.21

Caga-Positive Strains of H. pylori may Play a Role in Pre-eclampsia and Poliabortivity through a Cross-Reactivity with Trophoblast Cells


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Background: The role of bacterial and viral infections in trophoblast diseases, such as pre-eclampsia and poliabortivity, has been extensively studied in the past few years. Interestingly, while trophoblast cells show an endothelial phenotypic profile, a study from our group has shown that antibodies anti-CagA cross-react with endothelial cells, possibly playing a role in some vascular diseases. We hypothesized that anti-CagA antibodies may recognize antigens of trophoblast cells, thus impairing their function.

Materials and Methods: Placenta samples were obtained from healthy women. Trophoblast cells were cultured for 72 hours in a medium containing increasing concentration of polyclonal anti-CagA antibodies (from 6 to 200 µg/mL). Binding of anti-CagA antibodies to trophoblast cells was verified through flow cytometry and immunofluorescence, while the invasive potential of these cells was assessed by using a membrane invasion culture system and measuring of MMP-2 activity. Trophoblast lysate was also prepared for immunoprecipitation using polyclonal anti-CagA antibodies.

Results: Anti-CagA antibodies recognized antigens of trophoblast cells of all samples, showing a dose-dependent binding, both at cytotoxicity and at immunofluorescence. Incubation of trophoblast cells with increasing doses of anti-CagA antibodies
significantly reduced their invasiveness. Furthermore anti-CagA antibodies specifically precipitated two high molecular weight proteins from trophoblast lysate.

**Conclusions:** This study shows, for the first time, that anti-CagA antibodies recognize antigens expressed on the surface of trophoblast cells, reducing their invasiveness ability. These data give biologic plausibility to the theory that CagA-positive strains of *H. pylori* may play a role in trophoblast-related diseases.

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**Abstract no.: P05.22**

**Autoimmune Thyroid Disease and *H. pylori* Infection**

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**Aim:** *Helicobacter pylori* infection may be associated with autoimmune thyroid disease (ATD). Our aim was to verify whether the overall *H. pylori* and the CagA-positive (*CagA+*) *H. pylori* infection could influence the systemic levels of thyroid hormones, autoantibodies to thyroglobulin (TGA) and microsomal peroxidase (MPA) and pro- and anti-inflammatory cytokines in patients with ATD.

**Methods:** We enrolled 44 patients with ATD (mean age 49 [range 18 to 75] years) and 70 controls without ATD matched for age, gender, and social class. *H. pylori* infection and CagA status were determined serologically. All patients and controls underwent thyroid echography and determination of fT3, fT4 (pg/mL), TSH (µ/mL), TGA, MPA (U/mL), IL-1β, IL-6, TNF-alpha, and IL-10 (pg/mL). Statistics was performed by Mantel-Haenszel chi-square test and Mann-Whitney U test.

**Results:** Fifteen patients (34.0%) and 14 controls (20.0%) were infected (p = .09, odds ratio (OR) = 2.07, 95% confidence interval (CI) 0.81–5.31); seven infected patients and eight infected controls were CagA+ (NS). The mean levels of fT4 in CagA+ and CagA–infected patients were 8.23 [standard deviation (SD) 1.89] and 7.18 (SD 1.05) (p = .05). The difference between the levels of TGA in infected and uninfected patients was almost significant (595.21 [SD 1043.57] vs 277.02 [SD 693.56], p = 0.173). TNF-alpha concentrations in CagA+ patients were significantly increased with respect to CagA–infected and uninfected patients (1.90 [SD 2.47] vs 0.46 [SD 0.49] and 0.51 [SD 0.66], p = .05 and p = .028, respectively). IL-10 levels were 157 (SD 61) and 124 (SD 73) in infected and uninfected patients (p = .042).

**Conclusion:** *H. pylori* infection may influence the inflammatory response of ATD patients.

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**Abstract no.: P05.23**

**H. pylori Infection is Negatively Associated with Reflux Esophagitis: A Cross-Sectional Case-Control Study of 5616 Health Check-up Koreans**

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**Background:** The relationship between *Helicobacter pylori* infection and gastroesophageal reflux disease remains controversial.

**Aim:** To investigate this relationship in a large Korean population-based study.

**Methods:** A cross-sectional case–control study of 5616 health check-up subjects undergoing upper gastrointestinal endoscopy was conducted (2808 cases with reflux esophagitis versus age- and sex-matched controls). *Helicobacter pylori* infection was determined by measuring anti-*H. pylori* IgG.

**Results:** The prevalence of *H. pylori* infection was significantly lower in subjects with reflux esophagitis than in controls (38.4% vs 58.2%, p < .001). After adjusting for the effects of smoking, alcohol, waist circumference, and body mass index *H. pylori* infection is negatively associated with reflux esophagitis [odds ratio (OR) 0.44, 95% confidence interval (CI) 0.39–0.49]. There was significant inverse correlation between the severity of esphagitis and the prevalence of *H. pylori* infection. Subjects infected with *H. pylori* showed a significant increase in the risk of gastric atrophy (OR 3.22, 95% CI 2.79–3.70).

**Conclusions:** *H. pylori* seropositivity was independently associated with the reduced risk for reflux esophagitis and correlates with the milder grade of esophagitis in Korean population, which suggest that *H. pylori* infection may play an inhibitory role for reflux esophagitis.

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**Abstract no.: P05.24**

**Persistency of *H. pylori* in Inferior Third of Esophagus Epithelium in Patients with GERD**

O. S. Rzhavicheva* and V. V. Tsukanov†

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**Aim:** To study prevalence of *Helicobacter pylori* in epithelium of inferior third of esophagus in patients with gastroesophageal reflux disease (GERD).

**Methods:** Sixty-three patients with GERD (48 men and 15 women) aged 23 to 52 years were examined. All subjects underwent upper digestive tract endoscopy with biopsy and description of esophagus changes on Los Angeles classifications (1994). Morphologic research of esophagus epithelium included microscopic examination after staining by hematoxylin and eosin, and also definition of *H. pylori* dissemination after Gimsa staining.

**Results:** The erosive esophagitis was determined in 90.5% patients. The metaplasia of esophageal inferior third epithelium
was diagnosed in 19.0% persons. *H. pylori* was recorded in 19.0% patients. *H. pylori* was observed in 91.7% (11 of 12) patients with Barrett esophagus and in 2.0% persons (1 of 51) without metaplasia of esophagus (*p* < .001).

**Conclusion:** *H. pylori* was found often in metaplasied epithelium of esophagus and can render the influence on development of this disease.

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**Abstract no.: P05.25**

**Relationship Between *H. pylori* Infection, Gastric Atrophy, and the Risk of Esophageal Squamous Cell Carcinoma in Germany**

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*Department of Gastroenterology, Hepatology and Infectious Diseases, Magdeburg, Germany; †Department of Surgery, Magdeburg, Germany; ‡Institute for Biometrics, Magdeburg, Germany; †Institute for Biometrics, Magdeburg, Germany

**Background:** Recent studies from Sweden and Japan have shown a positive association between gastric atrophy and an increased risk for developing esophageal squamous cell carcinoma (OSCC). However, this findings need to be confirmed in other ethnic groups due to the wide geographic variation of this cancer and the changing prevalence of the *Helicobacter pylori* infection.

**Aim:** To investigate whether *H. pylori* infection and gastric atrophy are associated with an increased risk for OSCC using a case–control study in Germany.

**Methods:** Fifty-eight consecutive patients (40 males and 18 females, mean age 65 ± 9 years) with OSCC, and 116 sex- and age-matched controls with dyspepsia, were enrolled prospectively. Antrum and corpus atrophy were evaluated by histology of biopsy specimens and serology. Pepsinogen (PG)-I level < 30 µg/mL and PGII/PGI ratio < 2.5 were indicative for corpus atrophy, gastrin (G)-17 values of <1 pmol/L were suggestive for the presence of antrum atrophy. Fastening serum was analyzed for G17, PGI, PGII, and *H. pylori* antibodies using specific EIA tests (GastroPanel; Biohit, Plc). Anti-CagA antibodies were analyzed by immunoblot assay.

**Results:** *H. pylori* infection, assayed by either serology or anti-CagA antibodies, was not associated with an increased risk for OSCC (*p* > .05, Fisher exact test). The presence of gastric corpus and/or antrum atrophy diagnosed by means of histology, PGI, PGII, and/or G1-7 was also not associated with an increased risk for OSCC (*p* > .05).

**Conclusions:** Neither *H. pylori* infection nor gastric atrophy was associated with an increased risk for OSCC.

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**Abstract no.: P05.26**

**Helicobacter Eradication Therapy in Idiopathic Parkinsonism with Anti-nuclear Antibody: Implications for Better Methods of Detecting Low Density Infection**


*Psychiatry and Psychological Medicine, Institute of Psychiatry, London, UK; †Department of Gastroenterology, King’s College Hospital, London, UK; ‡Laboratory of Gastrointestinal Pathogens, Centre for Infections, Health Protection Agency, London, UK; §Statistics Unit, Centre for Infections, Health Protection Agency, London, UK

**Background:** Apparent *Helicobacter* eradication (to level of polymerase chain reaction on paired biopsies) converted malignant idiopathic parkinsonism (IP) to benign, marked deterioration accompanied proven failure (*Helicobacter* 2008;13:309–22).

**Methods:** Antinuclear antibody (ANA) was sought, before and 1.5 years after anti-*Helicobacter* therapy in IP probands (sera processed in single batch, blind to sequence). Duplicate measurements of primary outcome mean—stride length were made before (two occasions) and post-therapy (≥ 6 weekly intervals). Any background antiparkinsonian medication was constant, the short-t1/2 levodopa an exclusion.

**Results:** The context is a quarter of 126 IP probands being ANA-seropositive. In the urea breath test positive (29.6%), neutrophil and lymphocyte counts were not higher, CD8+ count was 28 [95% confidence interval (CI) 6, 54)%.

**Discussion:** Prognostic indicators are needed because *Helicobacter* is difficult to eradicate in IP, even when antimicrobial sensitivities are known/compliance monitored (*Helicobacter* 2008;13:309–22). Reduced cytotoxicity may impair clearance of residual organisms. ANA may alert to continuing low-density infection not detected by PCR on only two biopsies.
HSCs. The analysis revealed that 13 pathways were upregulated and 22 pathways were downregulated by microRNA. Furthermore, mitochondrial integrity based on highly upregulated Bcl-2 and downregulated caspase 3, 9 was confirmed in HSCs and fibrotic livers by immunoﬂuorescence assay, semiquantitative RT-PCR, qRTPCR, and Western blot. These ﬁndings provide in vitro and in vivo evidence that the mitochondrial pathway of apoptosis plays a signiﬁcant role in the progression of liver ﬁbrogenesis via HSCs activation.

Abstract no.: P05.28
The Prevalence of H. pylori Infection in the Patients with Different Gastroesophageal Reflux Disease Groups

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Aim: To study the prevalence of Helicobacter pylori infection in patients with GERD belonging to different age groups.

Material and Methods: A total of 722 patients were investigated: 326 patients older than 60 years and 244 patients under 60 years with GERD; the control group consisted of 152 patients without GERD. The severity of esophagitis was evaluated using the Los Angeles classiﬁcation. The diagnosis of H. pylori was carried out with histologic method and rapid urease test.

Results: H. pylori infection in GERD patients is revealed more seldom than in the control group. The chances ratio (CR) of association of H. pylori infection and GERD in patients older than 60 years were 0.28 (95% CL: 0.10–0.43), p = .00001, in the patients under 60 years it was – 0.59 (95% CL: 0.14–1.03), p = .0001. The analysis of clinical forms of GERD revealed that infection was the least in Barrette esophagus (BE) in both age groups. The analysis shows that in young patients the prevalence of H. pylori was higher in ERD, in elderly patients – in NERD. In increasing severity of esophagitis in the elderly, decrease of helicobacteriosis spreading was noted, while in young patients the spread of helicobacteriosis increased.

Conclusion: Level of helicobacteriosis not depending on age had distinct reverse connection with the presence of BE in patients of elderly group with severity of GERD.

P06 Molecular Genetics and Genomics, Virulence Factors and Pathogenesis I

Abstract no.: P06.01
Prevalence of cagA and jhp0947 Genes in H. pylori Isolates from First-Degree Relatives of Gastric Cancer Patients

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Introduction: Helicobacter pylori gastritis is a dynamic and progressive process. Studies suggest that a combination of different virulence genes determine the severity of inﬂammation. The aim of this study was to investigate the prevalence of cagA and jhp0947 genes in the ﬁrst-degree relatives of gastric cancer patients (FDRCPs) and their correlation with different types of gastritis.

Methods: One hundred and forty-three H. pylori strains were isolated from antral gastric biopsies of FDRCPs. All of the patients had gastritis. Three types of gastritis according to pathologic ﬁndings were: antral-predominant gastritis, corpus-predominant gastritis, and pangastritis. Genomic DNAs from isolates were subjected to PCR-based genotyping of cagA and jhp0947 genes. Primers were designed for ampliﬁcation of these genes.

Results: The prevalence of the cagA and jhp0947 among 143 H. pylori isolates from FDRCPs.

<table>
<thead>
<tr>
<th>Type of gastritis</th>
<th>Antral predominant</th>
<th>Corpus predominant</th>
<th>Genotype status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pangastritis</td>
<td>54 (73)</td>
<td>38 (51.4)</td>
<td>CagA positive</td>
</tr>
<tr>
<td></td>
<td>50 (78.1)</td>
<td>35 (54.7)</td>
<td>jhp0947 positive</td>
</tr>
<tr>
<td></td>
<td>5 (100)</td>
<td>1 (20)</td>
<td>Total</td>
</tr>
<tr>
<td>54 (73)</td>
<td>50 (78.1)</td>
<td>5 (100)</td>
<td></td>
</tr>
<tr>
<td>38 (51.4)</td>
<td>35 (54.7)</td>
<td>1 (20)</td>
<td></td>
</tr>
<tr>
<td>74 (51.7)</td>
<td>64 (34.4)</td>
<td>5 (3.6)</td>
<td></td>
</tr>
</tbody>
</table>

Discussion: cag A-positive and jhp0947-positive H. pylori strains were predominant in FDRCPs. However, we could not ﬁnd any association between genotypes of H. pylori strains and different types of gastritis. Although a number of putative H. pylori virulence genes have been associated with risks of a clinical outcome, none have clearly been linked to one speciﬁc H. pylori-related disease. Further studies with more isolates and candidate genes might help to predict the clinical outcome of H. pylori infection with certain genotypes.
HSCs. The analysis revealed that 13 pathways were upregulated and 22 pathways were downregulated by microRNA. Furthermore, mitochondrial integrity based on highly upregulated Bcl-2 and downregulated caspase 3, 9 was confirmed in HSCs and fibrotic livers by immunoﬂuorescence assay, semiquantitative RT-PCR, qRT-PCR, and Western blot. These ﬁndings provide in vitro and in vivo evidence that the mitochondrial pathway of apoptosis plays a signiﬁcant role in the progression of liver ﬁbrogenesis via HSCs activation.

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P06 Molecular Genetics and Genomics, Virulence Factors and Pathogenesis I

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Introduction: Helicobacter pylori gastritis is a dynamic and progressive process. Studies suggest that a combination of different virulence genes determine the severity of inﬂammation. The aim of this study was to investigate the prevalence of cagA and jhp0947 genes in the ﬁrst-degree relatives of gastric cancer patients (FDRCPs) and their correlation with different types of gastritis.

Methods: One hundred and forty-three H. pylori strains were isolated from antral gastric biopsies of FDRCPs. All of the patients had gastritis. Three types of gastritis according to pathologic ﬁndings were: antral-predominant gastritis, corpus-predominant gastritis, and pangastroitis. Genomic DNAs from isolates were subjected to PCR-based genotyping of cagA and jhp0947 genes. Primers were designed for ampliﬁcation of these genes.

Results: The prevalence of the cagA and jhp0947 among 143 H. pylori isolates from FDRCPs.

<table>
<thead>
<tr>
<th>Type of gastritis</th>
<th>Pangastritis</th>
<th>Antral predominant</th>
<th>Corpus predominant</th>
<th>Genotype status</th>
</tr>
</thead>
<tbody>
<tr>
<td>54 (%73)</td>
<td>50 (%78.1)</td>
<td>5 (%100)</td>
<td>CagA positive</td>
<td></td>
</tr>
<tr>
<td>38 (%51.4)</td>
<td>35 (%54.7)</td>
<td>1 (%20)</td>
<td>jhp0947 positive</td>
<td></td>
</tr>
<tr>
<td>74 (%51.7)</td>
<td>64 (%44.7)</td>
<td>5 (%3.6)</td>
<td>Total</td>
<td></td>
</tr>
</tbody>
</table>

Discussion: cag A-positive and jhp0947-positive H. pylori strains were predominant in FDRCPs. However, we could not ﬁnd any association between genotypes of H. pylori strains and different types of gastritis. Although a number of putative H. pylori virulence genes have been associated with risks of a clinical outcome, none have clearly been linked to one speciﬁc H. pylori-related disease. Further studies with more isolates and candidate genes might help to predict the clinical outcome of H. pylori infection with certain genotypes.
Abstract no.: P06.02
Prevalence of cagA and jhp0947 Genes in H. pylori Isolates from Patients with Gastritis, Gastric Ulcer, and Gastric Cancer

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Introduction: Helicobacter pylori plays an important role in the development of peptic ulcers, gastric adenocarcinoma, and gastric MALToma. Strains that possess the cag PAI/cagA are more associated with peptic ulcer and gastric cancer. The jhp0947 gene, located in the plasticity region of H. pylori genome, could report to be associated with an increased risk of both duodenal ulcer and gastric cancer. In this study prevalence of cagA and jhp0947 genes in Iranian H. pylori isolates and their relationship with gastrointestinal disorders were evaluated.

Methods: Seventy-five H. pylori strains were isolated from antral gastric biopsies. DNA was extracted and primers were designed for amplification of cagA and jhp0947 genes. PCR was performed and the products were electrophoresed.

Results: cagA+ was the predominant genotype among H. pylori isolates from all three groups of patients: ulcer (%76.2), gastritis (%78.5), and cancer (%40). The cagA+, jhp0947+ genotype was more prevalent: ulcer (%59.5), gastritis (%46.4), and cancer (%20).

Discussion: Until now no clear association has been found between host genetics, environmental factors, and H. pylori genotype and the gastric diseases. In this study no association was found between H. pylori genotypes and type of gastric disease. Further studies on H. pylori isolates from different groups of patient are needed to find more candidate genes involved in pathogenicity of H. pylori.

Abstract no.: P06.03
H. pylori Induces β3GlcNAc-T5 in Gastric Epithelial Cells Leading to Expression of Sialyl-Lewis X, the Ligand for SabA Adhesin

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Helicobacter pylori can produce phenotypic alterations in gastric epithelial cells. Expression of the inflammation-associated sialyl-Lex antigen in the gastric epithelium is induced during persistent H. pylori infection, suggesting that H. pylori may trigger the host tissue to re-tailor the gastric mucosal glycosylation patterns to a more favorable environment for its adhesion. H. pylori has been shown to adhere to glycoconjugates expressed in the gastric mucosa through bacterial adhesins (BabA, SabA). We evaluated the epithelial cells gene expression in response to H. pylori infection. Our results showed that H. pylori induced significant alterations in 168 of the 1031 genes tested in a microarray platform. The most virulent H. pylori strain led to altered expression of glycosylation-related genes, such as increased expression of β3GlcNAcT5, a glycosyltransferase involved in the synthesis of Lewis antigens. Further evaluation of a panel of H. pylori strains showed that induction of β3GlcNAcT5 expression was elicited only by the virulent H. pylori strains (cagPAI+). β3GlcNAcT5 overexpression in stably transfected gastric cell lines leads to increased expression of sialyl-Lex antigen and increased adhesion of H. pylori. In conclusion, our results show that highly pathogenic H. pylori strains induce β3GlcNAc-T5 and sialyl-Lex expression, the receptor for SabA adhesion, contributing to a successful infection. [1] Marcos NT et al. J Clinical Investigation 2008, 118:2325–2336.

Abstract no.: P06.04
FUT2-Null Mice Show Impaired BabA-Mediated Adhesion of H. pylori to Gastric Mucosa

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Helicobacter pylori adhesion is mediated by glycoconjugates expressed on gastric epithelial cells and constitutes a crucial step in the establishment of a successful infection. The blood group antigen-binding adhesin (BabA) binds to Lewis and H type-1 structures on gastric mucins, while a sialic-acid binding adhesin (SabA) recognizes sialylated carbohydrates mediating the adherence to inflamed gastric mucosa. Inactivating mutations in human FUT2 (secretor) gene are associated with reduced susceptibility to H. pylori infection. In this study we have used an animal model of non-secretors, FUT2-null mice, to evaluate the adhesion of H. pylori strains with different adhesins expression profile to the gastric mucosa of FUT2-null mice in comparison with the C57Bl/6 wild-type mice.

We have demonstrated that FUT2-null mice showed marked alteration in gastric mucosa glycosylation, characterized by diminished expression of α(1,2)fucosylated structures as indicated by lectins and antibodies staining. We further analyzed whether these modifications would have a role in H. pylori adhesion. H. pylori 17875/Leb strain (BabA+), that only expresses a functional BabA adhesion, bound to the foveolar epithelium of wild-type mice but no adhesion was observed in FUT2-null mice, while 17875babA1A2 strain (BabA−/SabA+) bound similarly the foveolar epithelium of both mice. The J99 strain (BabA+/SabA+) bound to both mice gastric mucosa, but adhesion levels were decreased in the FUT2-null mice. We further evaluated the adhesion of a panel of strains from clinical isolates that were characterized for BabA and SabA expression, showing that BabA-dependent adhesion was impaired in the FUT2-null mice, whereas SabA-mediated binding was not affected.

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Abstract no.: P06.05

Cloning and Expression of H. pylori Type IV Secretion System in Escherichia coli Cells by Detection of the Hummingbird Phenotype in Cultured Infected AGS Cells

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The Helicobacter pylori type IV secretion system (T4SS) is encoded by the 40 kb Cag pathogenicity island (PAI) which contains approximately 30 genes, including cagA. This system delivers the bacterial CagA protein into the cytoplasm of the host epithelial cell, promoting chronic infection and late gastric cancer. This T4SS has been characterized previously based on its similarity to the Agrobacterium tumefaciens T4SS. However, only a few components of the H. pylori T4SS have been identified, and it is thus defined as an incomplete system. To determine if genes located outside the PAI participate in the secretion system, the whole H. pylori 399 island was cloned and its functionality evaluated in Escherichia coli. Chromosomal fragments were ligated into a PeplFOS-5 fosmid and transferred to E. coli DH5α. Subsequently, a single clone carrying the entire island was isolated. The cagA encoded by PAI presented a deletion and was complemented with a plasmid carrying cagA from the strain G27. T4SS functionality was evaluated by inducting the “hummingbird” elongated phenotype of E. coli-infected AGS cultured cells. E. coli cells carrying the PAI, and complemented with the G27 cagA gene, induced this phenotype. The results showed that, after 48-hour infection, 30% of AGS cells displayed the “hummingbird” phenotype compared to 45.5% obtained after H. pylori G27 infection and 5% for uninfected control cells. These results suggest that cagPAI genes operate in E. coli cells and that CagA is translocated and phosphorylated inside AGS cells.

Funded by CTU06 Biomedicina Area 5 and Fondecyt 1085232 grant projects.

Abstract no.: P06.06

Helicobacter suis Induces Epithelial Cell Death Both in vivo and in vitro

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Helicobacter suis colonizes the stomach of more than 60% of slaughter pigs. Moreover, it is the most prevalent gastric non-H. pylori Helicobacter species in humans. Recently, this bacterium has been isolated in vitro from the stomach mucosa of slaughter pigs. Little is known about the virulence mechanisms of this and other gastric non-H. pylori Helicobacter species. Therefore, mice of two strains (BALB/c and C57BL/6) and Mongolian gerbils were inoculated intragastrically with this Helicobacter species. Transmission electron microscopy revealed the presence of H. suis in close contact with necrotic gastric epithelial cells, mainly parietal cells. Moreover, H. suis colonization was associated with an increased proliferation of mucosal epithelial cells. Both findings suggest a role for H. suis in the loss of gastric epithelial cells. In vitro, two gastric epithelial cell lines (human-derived AGS and mouse-derived GSM06 cells) were treated with sonicated H. suis. In both cell lines, these whole bacterial cell lysates induced cell death. Fluorescent staining with propidium iodide and antibodies directed against activated caspase-3 showed that both necrosis and apoptosis were present. Pretreatment of whole bacterial cell lysates with heat and trypsin abolished the cell-death-inducing capacity, allocating one or more proteins as the causative agent(s). Further studies should be undertaken to reveal the exact underlying mechanism.

Abstract no.: P06.07

Study of the Lipopolysaccharide of H. pylori Gastric MALT Lymphoma-Associated Strains: A Link with Lymphoma Pathogenesis?

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The aim of this project was to investigate the Lewis antigen expression in H. pylori gastric MALT lymphoma-associated strains in comparison to chronic gastritis only strains. Forty MALT strains (19 cagPAI (−) and 21 cagPAI (+)) and 39 gastritis strains (17 cagPAI (−) and 22 cagPAI (+)) were included in this study. The LPS for each strain was extracted using hot phenol method and the expression of Lewis X and Lewis Y antigens were investigated using Western Blot. The data were analyzed according to the strains’ cagPAI status and vacA genotype.

Lewis X antigens were identified in 21 MALT strains (52.5%) and Lewis Y in 30 strains (75%). Lewis X antigens were identified in 29 gastritis strains (74.3%) and Lewis Y in 31 strains (79.5%). There was an association between cagPAI status and Lewis X expression among MALT strains (p < .0001), but not in gastritis strains (p = .64).

Considering the disease status, among cagPAI (−) strains, the majority of gastritis strains (64.7%) were both Lewis X and Y positive, whereas the majority of MALT strains (63.2%) were Lewis Y positive only: strains expressing solely Lewis Y were associated with MALT (odds ratio = 64.2 (4.9–841.0)). No such association was found in cagPAI (+) strains. vacA genotypes did not modify the association between Lewis and disease status.

In conclusion, Lewis X antigens in cagPAI (+) MALT strains could participate to underbalance their proinflammatory effect. cagPAI (−) MALT strains have a particular Lewis antigen profile which could represent an adaptive mechanism to the host response.
Abstract no.: P06.08
The Intermediate Allele of vacA and the Vacuolation Induced by s1m2 Genotypes of H. pylori Strains

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Introduction: Several studies have previously demonstrated the s1m2 genotype as the predominant vacA genotype among circulating Helicobacter pylori strains in Iran. The functional toxicity of such vacA genotypes was not however determined. On the other hand, introduction of intermediate region of vacA provided new insights into the structural toxicity of vacA. The current study aimed to evaluate whether the intermediate region is involved in the toxicity of s1m2 vacA genotype strains.

Methods: A total of 109 H. pylori single-colony strains were studied. The multiplex polymerase chain reaction (PCR) of s and m vacA alleles were followed by i vacA PCR. Concentrated culture filtrates (CCF) of all of the studied strains were collected through liquid culture. HeLa cell line was incubated with 1:5 dilution of CCF up to 24 hours. The number of vacuolated cells was determined by inverted light microscopy. The statistical analysis was performed using Mann–Whitney test.

Results: In this study 27.5%, 1.8%, and 29.4% of studied strains were typed as s1i1m1, s1i2m1, and s2i2m2, respectively. Typing of the remaining strains showed that 19.3% were s1i1m2 genotype, whereas 22% were s1i2m2. Having studied the toxicity of s1m2 strains, we showed that number of vacuolated cells incubated with s1i1m2 CCF was significantly greater than that of s1i2m2 strains (p < .001).

Conclusion: This study suggested that intermediate allele is informative as toxicity assay and i vacA typing is able to classify more toxic types of s1m2 strains. This study confirms the application of such typing in substitution of the time consuming and laborious cytotoxicity assay.

Abstract no.: P06.09
Distinct Presence of CagA-Positive Strains with Higher Number of EPIYA-C Repeats in the Fundus Versus the Antrum of H. pylori-Infected Patients

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The EPIYA tyrosine phosphorylation sites are important determinants of CagA virulence presenting extensive variability in both the type and the number, especially the EPIYA-C sites, among wild-type H. pylori of Western origin.

We have previously identified the occurrence of mixed infections by isogenic H. pylori strains with variable number of EPIYA-C repeats within the same patient and we aimed to assess whether they preferentially colonize distinct compartments of the gastric mucosa, namely the gastric antrum and fundus.

CagA and EPIYA status were determined by polymerase chain reaction (PCR) and sequencing in H. pylori isolates from 140 paired antral and fundic biopsies from 70 Greek adult patients. Clonal relations between strains were assessed by RAPD-PCR and MLST analysis of the housekeeping genes atpA, efp, mutY, ppa, trpC, ureA, vacA, and yphC.

In all cases with the exception of one, paired isolates of antral and fundic origin were clonally related. In 59 patients, the same strain was isolated from both the antrum and the fundus (20 cagA-negative, 23 ABC, 11 ABCB, and 5 ABC/ABCC isolates), whereas in 10 patients the fundus was colonized by cagA-positive H. pylori harboring more EPIYA-C repeats compared to the corresponding strains from the antrum. In conclusion, with regard to the CagA EPIYA status, the vast majority of individuals were found to be infected by the same H. pylori strain. Nevertheless, in approximately 15% of the patients, isogenic strains carrying more EPIYA-C repeats were identified, preferentially colonizing the gastric fundus, possibly reflecting a response to microenvironmental differences in acidity between the two gastric sites.

Abstract no.: P06.10
CagA and VacA Polymorphisms are Associated with Distinct Pathologic Features in H. pylori-Infected Adults with Ulcer and Nonulcer Disease

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CagA and VacA polymorphisms define Helicobacter pylori virulence and may predict the development of severe gastric disease. We determined the variability of functional EPIYA tyrosine phosphorylation motifs in CagA and the isotypes for signal, intermediate, and middle regions of VacA in Greek adults with duodenal (n = 44) or gastric (n = 21) ulcers and nonulcer dyspepsia cases (n = 79) and assessed potential associations to the severity of histopathology.

EPIYA motifs were determined by polymerase chain reaction (PCR) and sequencing and VacA alleles by PCR. cagPAI functionality was assessed by interleukin (IL)-8 secretion, whereas CagA translocation was confirmed by Western blot detection of CagA, after antiphosphotyrosine immunoprecipitation of total protein lysates from H. pylori-infected AGS cells. Statistical analysis was pursued with multivariate logistic regression. Infection with CagA-positive strains carrying one EPIYA-C site was found to be an independent risk factor for gastroduodenal ulceration [odds ratio (OR): 4.647, 95% confidence interval (CI): 2.037–10.602], while the risk was 2-fold higher in mixed infections with isogenic strains harboring increasing EPIYA motifs. CagA species with
more EPIYA-C repeats exhibited higher tyrosine phosphorylation rates but did not contribute to elevated IL-8 secretion, or to increased neutrophil or mononuclear infiltration in the antrum. Increasing EPIYA-C repeats in cagA were associated with highly vacuolating vacA isotypes (s1/i1/m1 or m2). VacAs1 allele was related to increased activity of chronic antral gastritis (OR: 3.319, 95% CI: 1.449–7.600) and the vacAII allele to greater chronic inflammatory infiltration (OR: 6.514, 95% CI: 2.298–18.878). In conclusion, CagA and VacA contribute to H. pylori infection in a coordinated manner, differentially affecting clinical phenotypes and the inflammatory response.

Abstract no.: P06.11
How H. pylori Deals with Nitrosative Stress. Searching for Novel Defense Systems

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The production of nitric oxide (NO) by the enzymatic activity of iNOS constitutes a weapon of the eukaryotic immune system to fight pathogens and plays a key role in host defense in most bacterial and parasitic infections. NO and derived reactive nitrogen species have a severe impact in the pathogen, causing protein and lipid nitrosylation, damage to iron centers, inactivation of transcription regulators, enzymes and ion channels, and DNA damage. However, successful pathogens are able to respond to such aggressions by eliciting several protective mechanisms that in the end allow them to elude the host response and cause disease.

The human pathogen H. pylori is known to elicit the immune response, through the activation of the iNOS enzyme, and is submitted to an additional source of NO that derives from the chemical decomposition of nitrite in the acidic stomach’s environment. Nevertheless the mechanisms by which H. pylori can resist nitrosative stress remain poorly understood.

To further elucidate the H. pylori response to nitrosative stress we have started an exhaustive screening of strains deleted in genes of unknown function. For each strain, we analyzed the growth inhibition that is caused by NO in comparison to the parental strain H. pylori 26695. Our results show that at least three mutants have higher growth sensitivity to nitrosative stress, which make them good candidates for more thorough studies aiming to clarify their actual role in NO protection.

Abstract no.: P06.12
Mixed Infection with Different cagA-Positive H. pylori Strains in Iranian Gastrointestinal Patients

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Introduction: Cytotoxin-associated gene product (CagA) displays a critical role in pathogenesis of Helicobacter pylori strains. Different investigations show that 60% of Western and approximately all of the East Asian H. pylori strains are cagA positive. Therefore, only the presence of H. pylori cagA+ strains in various geographic regions with high prevalence of infection fails to be informative. Diversity in the 3’ region of this gene leads to varying virulent strains associated with more severe diseases. In this study we investigated H. pylori cagA subtype status and the presence of multiple infections among Iranian gastrointestinal patients.

Methods: Totally, 166 H. pylori-infected patients including 29 gastric adenocarcinoma, 32 peptic ulcer disease, 91 nonulcer dyspepsia, and 14 normal were enrolled. cagA diversities were determined in 466 H. pylori isolates from at least two biopsy specimens from different locations of the stomach. Polymerase chain reaction amplification was performed using primers cag2F/cag4R. SPSS package was used for data analysis.

Results: Collectively, 50% of the examined patients suffered from multiple infections (harboring more than one cagA subtypes). Of these 166 patients, 466 isolates were recovered which produced 563 H. pylori strains of which 513 were cagA-positive in nine different categories with the following distribution: 400 bp (1.0%), 450 bp (5.3%), 500 bp (2.9%), 550 bp (58.3%), 600 bp (2.1%), 650 bp (26.5%), 750 bp (3.3%), 800 bp (0.2%), and 850 bp (0.4%).

Conclusion: This investigation points to the remarkable fact that half of Iranian dyspeptic patients are infected with more than one cagA subtype strain of H. pylori which cautions against the use of this genotyping technique in identifying high-risk patients.

Abstract no.: P06.13
Detection of CagA EPIYA Motifs in H. pylori DNA Extracted from Recently Collected, Frozen, or Deparaffinized Biopsies and Clinical Samples

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CagA is a major virulence factor of Helicobacter pylori that, once injected into the epithelial cells and phosphorylated on specific bacterial tyrosine residues within repeating EPIYA-A, -B, -C, and -D motifs, localizes to the plasma membrane and interacts with a number of intracellular effectors suggested to play an important role in Helicobacter pylori pathogenesis. EPIYA-D (in East Asian CagA) and EPIYA-C motifs (in Western CagA) are the main sites of CagA phosphorylation and the presence both of EPIYA-D or an increasing number of EPIYA-C motifs, rather than the general CagA positivity, has been associated with more severe gastroduodenal disease.

With the aim to analyze EPIYA motifs in 24 cagA+ H. pylori isolates and in a number of recently collected, frozen, or, in particular, deparaffinized biopsies and clinical samples, all obtained from 62 patients with different H. pylori pathology, we comparatively evaluated EPIYA profiles by polymerase chain reaction amplification using single sets of primers flanking the variable EPIYA coding region, or a single forward primer and multiple reverse primers specific for the individual EPIYA motifs. Only the primers originally employed by Rudi et al. (1988) to amplify the variable 3′ region of the cagA gene allowed identification of EPIYA motifs in all biopsies and clinical samples. Multiple infections and EPIYA
profiles with more than one EPIYA-C motif, in some cases confirmed by DNA sequencing, were observed in 12 and 22 patients, respectively. As expected, the increasing numbers of EPIYA-C motifs were associated with more severe gastric pathologies.

Abstract no.: P06.14
Relationship Between hrgA Gene of H. pylori Infection in Gastritis and Peptic Ulcer Disease in Thailand

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Background and Aims: It has been identified that the putative Helicobacter pylori virulence factor, hrgA gene, might be a disease-specific marker for significant upper gastrointestinal tract diseases such as gastric cancer and peptic ulcer disease (PUD) in East Asian countries. Our aim was to study the relationship between hrgA gene of H. pylori infection and PUD in Thailand.

Methods: A total of 218 dyspeptic patients who underwent upper gastrointestinal endoscopy at Thammasat University Hospital, Thailand, during January 2007 to February 2008 were enrolled in this study. Two antral gastric biopsies were obtained for culture and hrgA/hpyIIIR status was determined by polymerase chain reaction using DNA expanded from a single colony.

Results: Forty-nine H. pylori-positive patients were enrolled in this study including 28 patients with gastritis and 21 patients with PUD. The mean age was 47.6 years (range 21–87 years). There were no significant differences in gender and age between patients with gastritis and PUD. hrgA gene was found in 39.3% in patient with gastritis and 42.9% in patients with PUD. However, the multivariate analysis did not identify statistical significant difference between these two groups (odds ratio = 1.2, 95% confidence interval: 0.4–3.6).

Conclusions: A direct relationship for hrgA gene and peptic ulcer disease could not be demonstrated in this study. Our data indicate that hrgA gene of H. pylori might not be associated with peptic ulcer disease in Thai patients.

Methods: We chose eight Japanese dupA-positive strains isolated in two distant areas with different risks of gastric cancer (Okinawa and Fukui), four from patients in Okinawa (two with duodenal ulcer (DU), two with gastric cancer (GC)) and as many from Fukui (two with DU, two with GC). Polymerase chain reaction (PCR) primers were designed to amplify a fragment corresponding to nucleotides in G27 (a dupA-positive strain deposited in GenBank), including jhp0917-0918 and those upstream. The PCR products were sequenced and compared with sequences of two other dupA-positive strains J99 and Shi470, deposited in GenBank.

Results: In Shi470, a strain isolated from Amerindian resident and related to East Asian strains, a continuous 2499 bp gene including dupA (HP_04615) were recognized. In Western strains, though G27 had a 2500 bp sequence homologous to HP_04615, it could not be a continuous gene by a stop codon. The upstream sequence to jhp0917-0918 was completely different from those of the former two strains in J99, the other Western strain. In this study, all eight Japanese strains sequenced here possessed a continuous 2499 bp gene homologous to HP_04615 (98.2–98.8%).

Conclusions: Our findings suggest that East Asian type of H. pylori strains have intact dupA. We could not find any significant difference in 2.5 kbp dupA region in strains from the two areas in Japan.

Abstract no.: P06.16
Influence of Chronobiologi Factors on Persistence of H. pylori

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Sufficiently expressed seasonal prevalence of exacerbation of gastroduodenal diseases (GDD) causes expediency of the analysis of influence of chronobiologic factors on Helicobacter pylori detection frequency and the semination degree by them of the organism in patients with GDD. The results of bacteriologic investigation of 514 samples of gastric juice of patients with duodenal ulcer were analyzed on H. pylori presence. Mid-annual frequency of H. pylori revealing made 73.7%. However this parameter depending on the month of investigation changed in essential limits: from 29.3% in August up to 88.5% in February. The presence of four peaks of H. pylori isolation was determined: February, March, September, and November, whereas minimal levels of the isolation frequency of these microorganisms fell in April, August, October, and December. Peaks of gastric juice semination with H. pylori were observed in January, June, and October. The minimal values of this parameter were noted in January and October. It is possible to note highly expressed parallelism of the studied parameters; their significant reduction in August and then sharp rise in September (p < .05) attract special attention. The most expressed growth of these parameters fell in the beginning of autumn and spring seasons: the periods of GDD exacerbation, and reduction in parameters was noted during the periods of recession of frequency of these diseases and their relapses. The presented data serve as additional arguments in support of H. pylori etiologic value at GDD, as rise of H. pylori semination and their isolation frequency precede GDD exacerbation growth, and not the opposite.
Abstract no.: P06.17
Identification and Characterization of CagA EPIYA Motifs in Turkish Origin Strains

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CagA is the most known virulence factor in Helicobacter pylori and increases the risk for disease. The aim of the study is to identify CagA EPIYA motifs in Turkish H. pylori strain and correlate with pathologic findings in patients’ biopsies.

Method: Endoscopies were performed in 62 adult patients. Antrum and corpus biopsies obtained for histology and culture. H. pylori strains were vacA genotyping and cagA status established as described (Helicobacter, 11, 2006). H. pylori cagA-positive polymerase chain reaction (PCR) products ranging from 370 to 570 bp were amplified (J Clin Microbiol, 45, 2007) and PCR products purified by QIAquick PCR kit (Qiagen) and sent to Macrogen (http://www.macrogen.com) for sequencing.

Results: We found 25 uninfected patients (41%) and 37 H. pylori-positive patients (59%). Uninfected patients had less chronic gastritis (p = .015) and were older than H. pylori-positive patients (p = .005). Among H. pylori-positive patients 17 (46%) were CagA positive. H. pylori CagA-positive patients had higher atrophy scores and intestinal metaplasia than H. pylori CagA-negative patients (p = .02). All H. pylori cagA-positive strains were vacA s1/m1 or s1/m2. In contrast, H. pylori cagA-negative strains were s2/m2 (75%). ABC EPIYA motif was common in Turkish strains (64.7%). However, no difference in pathology associated with number of EPIYA motifs was observed. Some patients (35.7%) were colonized with more than one strain (mix infection) based on phylogenetic analysis and variation in vacA and/or cagA genotype.

Conclusion: We confirmed the low prevalence of CagA-positive strains among Turkish patients and predominant ABC type in EPIYA motifs.

Abstract no.: P06.18
Associations of the Plasticity Region of H. pylori in Patients with Gastroduodenal Diseases

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The virulence genes of H. pylori outside cag pathogenicity island are described as plasticity region. The plasticity region genes include JHP0940, JHP0947, and JHP0986. The first two are associated with an increased risk of duodenal ulcer and gastric carcinoma (GC) while later with gastritis. We determined distribution of JHP0940, JHP0947, and JHP0986 and cagA in H. pylori isolates from patients with gastroduodenal diseases.

Methods: Of 43 isolates, 35 patients had gastritis, six peptic ulcers (PU) (four duodenal ulcers, two gastric ulcers), and two gastric carcinoma. DNA was extracted, and polymerase chain reaction was done for JHP0940, JHP0947, JHP 0986, and cagA gene using primers described before. Differences in proportion were assessed by Pearson chi-square, Fisher exact, or likelihood ratio test where appropriate. p value < .05 was significant.

Results: Of 43 patients, 29 (67%) were male, mean age 41 ± 13 years, range 22–69. JHP0940 was positive in 15 (35%), JHP 0947 in 14 (33%), JHP0986 in 10 (23%), and cagA in 21 (49%). JHP0986 was associated with cagA in four (40%) (p = .52), JHP0940 in seven (47%) (p = .83), and JHP0947 in nine (64%) (p = .16). JHP0947 was positive in five (83%) with PU, one (50%) with GC, and eight (30%) with gastritis (p = .02); JHP0940 was positive in two (100%) with GC, two (33%) with PU, and 11 (31%) with gastritis (p = .11) with GC and JHP0986 was associated with only gastritis in nine (26%) and with PU in one (17%) (p = .51).

Conclusion: JHP0947 gene was associated with peptic ulcer and gastric carcinoma. There was no association of cagA with hyper-plasticity region genes; however, these are preliminary results and study is ongoing.

Abstract no.: P06.19
Detection of H. pylori vacA and cagA Virulence Genotypes by PCR

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Prognosis of the Helicobacter pylori infections is strongly associated with the bacterial virulence factors. The most important virulence factors are proteins which are encoded by cagA and vacA gene. Generally, the presence of cagA and distribution of vacA allelic types have shown considerable geographic variation. The aim of this study was to determine the prevalence of cagA and vacA allelic types and to evaluate the association between clinical findings. The cetyltrimethyl-ammonium bromide (CTAB) method was used for the extraction of the DNA templates. In order to confirm the presence of H. pylori in stock strains, a 411-bp fragment of the ureA gene was amplified by polymerase chain reaction. After that, 120 isolates that are positive for ureA gene were amplified by primers which target specific sequences of vacA and vacA alleles (s1a, s1b, s2, m1, m2). The cagA were detected in 64 (53.3%) of the 120 strains. The s1a, s1b, and s2 variants were detected in 70.1%, 2.8%, and 27.1%, respectively. Among middle(m) region variants, m2 (65.5%) was found more prevalent than m1 (33.6%). Both m1 and m2 genotypes were found together in one strain. The most frequently seen allelic combination was s1a/m2 (35.6%) and s1a/m1 (33.6%). Furthermore, no strain with the s2/m1 combination was found. Although, there was no significant association between cagA positivity, vacA alleles, and clinical findings, an association between vacA allelic combinations and cagA positivity has been detected.
Abstract no.: P07.01

H. pylori vacA Intermediate Region i1 Strains are Associated with More Severe Histologic Features of Chronic Gastritis and Increased Gastric Carcinoma Risk in Portugal

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 Abstract no.: P07.02

H. pylori CagA+ Infection and Serum Pepsinogen Concentrations in Portuguese Patients with Gastric Carcinoma and Advanced Precancerous Lesions in a High-Risk Population in Costa Rica

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 Abstract no.: P07.03

Long Term Follow up of the Low Grade Gastric Dysplasia


Despite the recognized importance of dysplasia as being the penultimate stage of gastric carcinogenesis, its natural history...
remains to be defined. In this study, we aimed to explore the long-term evolution of low-grade dysplasia which was not suitable for primary endoscopic treatment. We have analyzed 32 cases of low-grade dysplasia, with a minimum follow-up of 12 months (mean 40.2 months). A statistical correlation (χ² test) was performed between the following variables: the progression of low-grade dysplasia (regression, maintenance, or progression of histologic grade); type of endoscopic appearance (flat, nodular, or depressed mucosa), and anatomical distribution. We found that 9.4% of patients were younger than 55 years and with family history of gastric cancer; 97% had extensive atrophy and intestinal metaplasia and 78% showed only antrum dysplasia. Concerning the endoscopic appearance, 50% showed dysplasia in flat mucosa, 41% nodular mucosa, and 9% revealed depressed mucosa. The histologic grade has regressed in 66% of cases (mean time of 9.1 months), unchanged in 28% and progressed in 6% (median time of 48 months). Regarding the lesions that have regressed, 15 of 21 (71.4%) were initially detected in flat mucosa (p < .008). The two cases (6%) which had progression of histologic grade were initially multifocal low-grade dysplasia, while the others showed no multifocal distribution (p < .001). Altogether our results demonstrated that almost all patients with low grade dysplasia had extensive atrophy/intestinal metaplasia. Furthermore, the majority of the patients showed regression of lesions in clinical follow up and 6% of the cases have progressed in the histological grade. Moreover we found that the initial detection of changes in flat mucosa significantly correlates with their regression. In addition the multifocality of the lesions was significantly correlated with its progression.

Abstract no.: P07.04
Biologic Role of Pteridium aquilinum in Gastric Epithelial Cells: Potential Synergistic Effect with H. pylori Infection

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Helicobacter pylori is associated with the development of gastric cancer. Gastric carcinogenesis is a multifactorial disease also related to host and environmental factors. Bracken fern (Pteridium aquilinum) is the only higher plant known to cause cancer naturally in animals. The carcinogenic toxin of bracken, ptaquiloside, was identified not only in the plant, but also in cow's milk and water. Epidemiologic studies have consistently shown an association between bracken exposure and gastric cancer.

The aim of this work is to evaluate the involvement of P. aquilinum in the process of gastric carcinogenesis potentiated by the presence of H. pylori.

We have prepared bracken fern extracts and evaluated alterations on gastric cell lines and on H. pylori strain 26695 treated with these extracts. We further exposed C57Bl/6 mice infected or not with H. pylori to P. aquilinum in order to identify histologic and molecular alterations at gastric mucosa level.

At the highest doses of bracken fern extract, we observed an inhibition of gastric cell lines proliferation and bacterial growth when cultured in vitro. In vivo, histomorphologic alterations on gastric mucosa, an increased proliferative index, and induction of DNA strand breaks as revealed by γH2AX immunolabelling were observed in mice stomach exposed to P. aquilinum whatever the infection status. We further evaluated mice gastric mucosa glycosylation alterations upon P. aquilinum exposition, observing an increased expression of sialylated structures.

These results suggest that P. aquilinum in association with H. pylori infection could have an important effect in the human gastric carcinogenesis process.

Abstract no.: P07.05
H. pylori Infection and Premalignant Mucosal Changes in the First-Degree Family Members of Gastric Cancer Patients

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Background/Aims: Relatives of gastric cancer patients have higher risk of gastric cancer. We evaluated the association of Helicobacter pylori infection and premalignant mucosal changes with family history of gastric cancer.

Patients and Methods: A total of 235 cases (first-degree family member of gastric cancer patients) and age, sex-matched controls (participants of national screening program for gastric cancer) were enrolled. Detection of H. pylori and histologic assessment for gastric mucosa was evaluated using updated Sydney system at antrum and at upper body lesser curvature (UBLC). Advanced histologic changes were defined as moderate or severe grades for glandular atrophy and intestinal metaplasia (IM). Multiple logistic regression method with odds ratios (ORs) and 95% confidence intervals (CIs) were used for analysis.

Results: Male-to-female ratio was 2.7:1 and median age was 57 years. H. pylori infection rates were 64% (150 of 235) in case and 62% (146 of 235) in control group [odds ratio (OR) = 1.07, confidence interval (CI) = 0.74–1.57]. Advanced atrophy rates at antrum were 57.9% in both groups (p > .99). Those figures at UBLC were 30.6% in case and 35.3% in control group (OR = 0.84, CI = 0.56–1.24). Advanced IM rates at antrum were 28.9% in case and 35.3% in control group (OR = 0.84, CI = 0.56–1.24). Advanced IM rates at antrum were 28.9% in case and 35.3% in control group (OR = 0.84, CI = 0.56–1.24). Advanced IM rates at antrum were 28.9% in case and 35.3% in control group (OR = 0.84, CI = 0.56–1.24). Advanced IM rates at antrum were 28.9% in case and 35.3% in control group (OR = 0.84, CI = 0.56–1.24). Advanced IM rates at antrum were 28.9% in case and 35.3% in control group (OR = 0.84, CI = 0.56–1.24).

Conclusions: H. pylori infection rate and advanced atrophy at gastric mucosa of the first-degree family members of gastric cancer patients were not significantly different from those of controls. Advanced IM at antrum was more frequent in the family members.
Abstract no.: P07.06
Increased Proliferation of CD4+FOXP3+ T Cells in Gastric Cancer Mucosa Contributes to Higher Numbers of Regulatory T Cells

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An increase of regulatory T cells, defined as CD25high and/or FOXP3+ expressing CD4+ T cells, within tumors has recently been reported in several studies. It has been suggested that these cells help the tumor to modulate the antitumor immune response mainly by inhibiting T-cell-mediated tumor cell killing in an interleukin (IL)-10- and/or transforming growth factor (TGF)-β-dependent way. In this study we confirmed increased levels of CD4+FOXP3+ T cells in gastric tumor mucosa of patients with gastric adenocarcinoma and show that CD4+FOXP3+ T cells in the tumor proliferate twofold more in situ than CD4+FOXP3+ isolated from tumor-free mucosa of the same patient. Furthermore, the CD4+FOXP3+ T cells in the tumor proliferated to a higher degree than CD4+FOXP3+ T cells in the same location. When CD4+ T cells were isolated directly from the tumor and tumor-free mucosa and sorted in CD25high and CD25low populations for gene expression analyses we found that CD4+CD25high T cells have a higher IL-10/interferon (IFN)-γ gene expression ratio but express lower levels of TGF-β than CD25low/-T cells. Analysis of the total gene expression in tumor and tumor-free mucosa revealed as expected an increase in FOXP3 expression in the tumors, but also markedly higher levels of both IFN-γ and IL-8.

In conclusion, we suggest that gastric tumors promote proliferation of regulatory CD4+FOXP3+CD25high T cells within its cell mass, and that the increased proliferation contributed to higher numbers of regulatory T cells in tumor tissues which by the resulting immunosuppression allows an advantage for the growing tumor cells.

Abstract no.: P07.07
The Presence of H. pylori on the Gastric Mucosa and Systemic Specific Antibodies May Influence the Outcome of Gastric Cancer After Surgical Resection

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Aim: Helicobacter pylori infection is a main determinant of gastric cancer (GC). The outcome of GC after surgical resection (SR) could be influenced by the presence of H. pylori in the stomach and specific antibodies (SA) at the time of SR.

Methods: The H. pylori status, SA response, and clinical outcome were investigated in a large cohort of patients. Frozen non-neoplastic gastric mucosa and serum samples were obtained from 297 patients who underwent surgery for primary GC between 1988 and 2004. H. pylori status was defined by means of polymerase chain reaction (PCR) analysis for the vacA gene in gastric mucosa and by serologic assay of H. pylori and CagA antibodies. Univariate and multivariate analyses were used for the association between clinic and pathologic variables and long-term outcome.

Results: Positivity for H. pylori infection was observed in 256 of 297 patients (86%), whereas in 41 patients (14%), PCR for vacA and both serologic tests were negative. Negative H. pylori status was found to be significantly associated with cardia location, advanced pT classification, noncurative surgery, and a lower 5-year survival rate after R0 resection (24% vs 57%, p < .001). Multivariate survival analysis confirmed H. pylori status as a significant prognostic factor [hazards ratio, 2.47; 95% confidence interval, 1.40–4.35 (p = .002)]. The influence of H. pylori status on long-term survival was observed in patients with early as well as advanced pT classifications.

Conclusions: Negative H. pylori status appears to be an indicator of poor prognosis in patients with gastric cancer, and is independent of other well-known clinical and pathologic prognostic variables.

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Abstract no.: P07.08
Notch Inhibition Overturns the Apoptosis Resistance Conferred by E-cadherin Deficiency

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Germline mutations of the E-cadherin gene constitute the molecular basis of hereditary diffuse gastric cancer (HDGC). The role of E-cadherin in tumourigenesis has been attributed to its ability to suppress invasion and metastization. However, E-cadherin impairment may have a wider impact on tumour development. We have previously shown that overexpression of human E-cadherin in Drosophila produces a phenotype characteristic of downregulated Notch. These observations led us to hypothesize that Notch signaling may be influenced by E-cadherin and mediate tumor development associated with E-cadherin deficiency.

E-cadherin-negative MDA-MB-435 cell line was transduced with wt E-cadherin or HDGC-related germline mutations T340A and V832M. De novo expression of wt E-cadherin led to a significant decrease in the activity of the Notch pathway. However, the mutated forms of E-cadherin did not exhibit the same ability to inhibit Notch-1 signaling. This increased Notch-1 activity correlated with increased expression of Bcl-2, an anti-apoptotic protein. In agreement with these findings, E-cadherin-deficient cells showed increased resistance to apoptotic stimuli when compared to wt E-cadherin cells. To confirm that such resistance was associated with increased Notch-1 activation, siRNA and the pharmacologic drug DAPT were used to block Notch-1 signaling. Under apoptotic stimuli, inhibition of Notch-1 resensitized E-cadherin-deficient cells to apoptosis in a similar degree to wt E-cadherin cells.
Our results show that aberrant Notch-1 activation, Bcl-2 expression, and cell survival are likely to play a crucial role in neoplastic transformation of E-cadherin-deficient cells. These findings suggest Notch inhibition as a possible therapeutic target for tumors associated with E-cadherin inactivation.

### Abstract no.: P07.09
**Gastric Cancer and H. pylori Infection in Thailand: A 10-Year Review**

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**Background and Aims:** Gastric cancer is the second leading cause of cancer death worldwide. This study was designed to evaluate the clinical, pathologic features, and molecular study of *Helicobacter pylori* infection in Thai gastric cancer patients.

**Methods:** Clinical information, histologic features, and *H. pylori* status were collected from all gastric cancer patients during June 2000 to May 2009. *H. pylori* isolates were genotyped by polymerase chain reaction (PCR) based on *cagA*, *vacA* right end region junction, and *vacA* genotypes.

**Results:** A total of 109 gastric cancer patients were enrolled in this study. Common presenting symptoms were dyspepsia (71%) and weight loss (69%). Mean duration of symptoms was 105 days. Overall prevalence of *H. pylori* infection was 83%. There was no difference between male and female (87.1% vs 78.6%; *p* > .05) and prevalence of *H. pylori* infection in diffuse type and intestinal type gastric cancer (82% vs 85%; *p* > .05). East Asian genotype (*cagA* 1a, *vacA* s1c, and/or *vacA* m1b) was highly prevalent in Thai gastric cancer patients (65%). East Asian genotype was more common in gastric cancer age ≤ 50 years than those patients age > 50 (74% vs 43%; *p* < .01) and more common in patients from city than those from rural area (79% vs 50%; *p* < .05).

**Conclusion:** Patients with gastric cancer in Thailand usually delay diagnosis and present at advanced stage with a 5-year survival less than 15%. *H. pylori* infection was highly associated with both intestinal type and diffuse type. East Asian genotype may be regarded an important factor for pathogenesis and prediction gastric cancer in Thailand.

### Abstract no.: P07.10
**H. pylori Infection and Gastric Cardia Cancer: Systematic Review and Meta-analysis**

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**Introduction:** *Helicobacter pylori* infection is the most important risk factor for non-cardia gastric cancer, but no association is recognized between infection and cardia cancer. However, two etiologically distinct types of cardia gastric cancer were recently proposed, one associated with *H. pylori*-induced atrophic gastritis and other occurring in non-atrophic gastric mucosa.

We quantified the association between *H. pylori* infection and gastric cardia cancer through meta-analysis, addressing the within- and between-study heterogeneity.

**Methods:** We systematically reviewed published articles on the association between infection and gastric cardia cancer. Studies were identified in published meta-analyses on the association between infection and gastric cancer (up to 2003) and in PubMed (from 2003 to April 2009). From each article we extracted estimates of the association between infection and cardia and non-cardia cancer.

Summary relative risk (RR) estimates and 95% confidence intervals (95% CI) were computed using random-effects models, and heterogeneity was quantified through the *I²* statistic. Stratified analyses were conducted for studies from low- and high-risk settings (gastric cancer age-standardized (world) incidence rate >18.0/100,000-GLOBOCAN 2002).

**Results:** For cardia cancer, crude RR was 0.98 (95% CI: 0.78–1.22; *P* = 62.7%; 35 studies), and the adjusted RR 1.05 (95% CI: 0.83–1.34; *P* = 53.5%; 25 studies). The adjusted RR was 1.59 in high-risk (95% CI: 1.26–2.02; *P* = 0%; 12 studies) and 0.76 in low-risk settings (95% CI: 0.62–0.94; *P* = 0%; 13 studies).

For noncardia cancer, the adjusted RR was 2.57 (95% CI: 1.59–4.17; *P* = 84.0%; 10 studies) in high-risk and 2.92 (95% CI: 2.29–3.71; *P* = 48.7%; 17 studies) in low-risk settings.

**Conclusion:** In high-risk settings the association with infection is similar for cardia and noncardia cancers.

### Abstract no.: P07.11
**Analysis of H. pylori Infection on MLH1 Promoter Methylation Status and Microsatellite Instability**


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*Helicobacter pylori* infection is a major gastric cancer risk factor. It is well known that DNA methylation is implicated in DNA mismatch repair genes deficiency. Deficient DNA mismatch repair caused by *H. pylori* may underlie microsatellite instability in the gastric epithelium and may represent a major mechanism of mutation accumulation in the gastric mucosa during the early stages of *H. pylori*-associated gastric carcinogenesis. Alterations in DNA mismatch repair (MMR) proteins result in microsatellite instability (MSI), increased mutation accumulation at target genes, and cancer development. Thus, the aim of the present study was to evaluate the influence of *H. pylori* infection on MLH1 promoter methylation status and its mRNA levels and, correlate them with microsatellite instability in patients with chronic gastritis and gastric cancer. The study included 217 patients of which 26 were uninfected; 127 had chronic gastritis and were *H. pylori* positive, and 64 had gastric cancer. Methylation status was evaluated by methylation-specific polymerase chain reaction. The expression levels were determined by quantitative real-time
polymerase chain reaction. MSI were analysed polymerase chain reaction at five loci according to the Bethesda criteria. H. pylori infection was associated with an overall increase in expression of MLH1 in patients with chronic gastritis. There was no correlation between H. pylori infection and the methylation status or MSI pattern. On the other hand, these levels decrease significantly among gastric cancer patients. In gastric cancers, the loss of expression of MLH1 was associated with its methylation status and MSI-high phenotype.

**Abstract no.: P07.13**

**Especially H. pylori-Positive Patients with Distal Gastric Cancer Have More Severe Local Intestinal Metaplasia**

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**Background:** This retrospective study aimed to characterize structural changes of the gastric mucosa and their location-specific differences in gastric cancer (GC).

**Materials and Methods:** Tumor location was assessed endoscopically for 130 patients with GC (64.9 ± 12.9 years). If the major tumor mass was located subcardial, in the fundus or the proximal one third of the corpus, carcinomas were classified as proximal (n = 51), as distal with the main tumor mass in the distal two thirds of the corpus or the antrum (n = 79). Seventy tumors were of the intestinal, 60 of the diffuse type. *Helicobacter pylori* status was assessed by serology. Mucosal alterations were classified according to the updated Sydney classification and consequently compared by localization, histologic type, and *H. pylori* status (Wilcoxon-, Mann–Whitney U-test).

**Results:** *H. pylori* prevalence was not different between proximal and distal GC (80.4% vs 77.2%). There was no association between *H. pylori* status and Laurén type. Proximal, there were more intestinal carcinomas than distal (66.7% vs 45.6%; p = 0.02). There was no difference in location-specific gastritis scores, except a higher degree of IM for distal intestinal type tumors compared to proximal GC (p = 0.045). Generally, IM was more severe in the antrum than the corpus, which was particularly confirmed for distal but not for proximal GC (p = 0.005). The effect was more evident for intestinal GC and positive *H. pylori* status (p = 0.003, < 0.001, respectively). Atrophy scores did not differ between the groups compared.

**Conclusion:** There is a higher background of tumor-surrounding IM in distal than proximal GC, especially for intestinal type tumors with positive *H. pylori* status, suggesting a different carcinogenic pathway for proximal and distal GC.

**Abstract no.: P07.14**

**Tumor Markers in H. pylori-Infected Patients**

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**Introduction:** The relationship between *Helicobacter pylori* infection and gastric cancer is one of the major importances in understanding the cancer process. The aim of this study was to investigate whether serum tumor markers (STM) can be used as prognostic indicators of gastric cancer under *H. pylori* infection.

**Methods:** Twenty-six patients with *H. pylori* infection confirmed by ELISA using ‘*H. pylori* IgG ELISA’ (“Biohit Plc.”, Finland) were evaluated for eight STM using proper “CanAg EIA Kit” (“CanAg
Abstract no.: P07.15
Mitochondrial Transition Pore and its Relationship with Apoptosis in Gastric Epithelial Cells Infected with *H. pylori*

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Introduction: During transduction of apoptotic signals into the cell, alterations as a consequence of “mitochondrial permeability transition pore (MPTP)” opening take place in mitochondrial membranes. MPTP is a nonspecific channel and appears to be involved in the translocation of pro-apoptotic components to the cytosol from mitochondria (cytochrome c) and vice versa (Bax). Continuous pore activation results from pro-apoptotic conditions such as oxidation of glutathione or free radicals oversynthesis.

Objective: To analyze the modifications that *Helicobacter pylori* causes in gastric epithelial cells at MPTP level, and the influence of antioxidant VitE and of Bax translocation inhibitor pentapeptide V5.

Materials and Methods: Human gastric epithelial cells (AGS, ATCC CRL-1739) infected with *H. pylori* (ATCC-51932) (10⁶ CFU/ml) were pretreated with V5 and VitE (100 µmol/L) for 24 hours. By confocal microscopy, it determined the following:
- Production of mitochondrial Q⁺ [MitoSOX Red(5 µmol/L)]
- Reduced glutathione [mBBr(µmol/L)]
- MPTP opening [calcine-AM(1 µmol/L)]
- Mitochondrial transmembrane potential (∆ψm) [JC-1 (2 µmol/L)]
- Number of apoptotic cells [AO(1 µmol/L)]

Results: *H. pylori* induced mitochondrial oxidative stress in AGS cells. Moreover, it decreased ∆ψm and caused MPTP opening, as well as apoptosis. VitE addition counteracted all these alterations.

The pretreatment with V5 also recovered the data of ∆ψm, MPTP opening, and number of apoptotic cells, compared to control.

Conclusions: The apoptotic processes caused by *H. pylori* take place through mitochondrial pathway by MPTP opening and ∆ψm dissipation, being the oxidative stress at the beginning of these changes. These events control the mitochondrial membranes permeability, and the pro-apoptotic protein Bax plays an active role. A strategy with antioxidants and/or Bax inhibitors could be considered in the clinical management of *H. pylori*-infected patients.

Abstract no.: P07.16
Effects of *H. pylori* Infection on Apoptosis in Patients with Chronic Gastritis


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Although *Helicobacter pylori* infection is considered to be one of the earliest steps in gastric carcinogenesis, the mechanisms by which the infection increases gastric epithelial cell proliferation and apoptosis need to be better comprehended. There is emerging evidence that apoptosis plays an important role in the pathogenesis of a variety of diseases. In the gastrointestinal mucosa apoptosis has an essential function in maintaining its integrity. Its deregulation is associated with the occurrence of lesions such as atrophic gastritis, peptic ulcers, intestinal metaplasia, and gastric cancer.

Thus, we evaluated the effects of *H. pylori* infection on apoptotic cells (apoptotic index, AI), and correlate the AI with Bax and Bcl-2 expression in patients with chronic gastritis. The study included 153 patients, of which 26 were uninfected; 127 had chronic gastritis and were *H. pylori*-positive. Bacterial genotypes were evaluated by polymerase chain reaction (PCR), the apoptotic index was investigated by means of TUNEL assay, and the expression values were determined by quantitative real-time PCR. Our data showed that the upregulatory effects of *H. pylori* infection on the pro-apoptotic gene, Bax, were stronger than its induction of Bcl-2. Similar results were observed in TUNEL assay, indicating an increase in AI in patients with chronic gastritis. Regarding the effect of virulence factors in apoptosis, an association was found between the infection with the *cagA*-positive *vacA* s1m1 strains and the higher expression and AI levels.

Abstract no.: P07.17
Long-Term Complete Remission of Gastric Diffuse Large B Cell Lymphoma After Eradication of *H. pylori*: A Retrospective Study of 10 Cases

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Gastric diffuse large B cell lymphomas (DLBCL) are believed to be *Helicobacter pylori*-independent. A few cases of remission after...
H. pylori eradication have been reported. Our aim was to report long-term follow up of 10 gastric DLBCL responding to H. pylori eradication.

From 1997 to 2007, 10 patients with H. pylori-positive gastric DLBCL, five males, five females, median age 57 years (range 34–75), were treated with H. pylori eradication. Seven were “de novo” DLBCL and three were DLBCL with MALT-type lymphoma component. Patients were in good condition and had gastric ulcers associated with gastric hemorrhage in three cases. They were localized lymphomas: 5 stage IE, 3 IIE1, and 2 IIE2. Endoscopic ultrasonography (n = 8) showed lymphoma infiltration reaching the submucosa in one, muscular layer in one, and serosa in six and was associated with lymphadenopathy in five. International prognostic index was 0 or 1. H. pylori was eradicated in all. Endoscopic follow up was performed every 4–6 weeks until complete response, then every 3–6 months for 1 year, then every year. The mean time to obtain complete response was 10.5 weeks (range: 10 days to 5 months). After a median follow up of 5 years (range: 2–11) all patients were alive and remained in complete remission.

This study shows that H. pylori eradication may induce durable complete response of gastric DLBCL. A complete response may be obtained even in case of deep lymphoma infiltration ± associated with lymphadenopathy and in the absence of MALT-type lymphoma component.

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**Abstract no.: P07.18**

Evaluation of Genotypes of *H. pylori* in Venezuela Patients from Endemic Gastric Cancer Andes County

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Eligible subjects were participants in the gastric cancer control program of Táchira State, Venezuela, between 25 and 69 years of age, who had been referred for gastroscopy at the Cancer Control Center of Táchira State in San Cristóbal. After they gave written informed consent, all subjects underwent gastroscopy examination with collection of gastric biopsies and blood. There were 120 eligible subjects.

Seven biopsies were taken. Five of the biopsies were for histologic assessment. Two biopsies were frozen at −80 °C until used for *H. pylori* genotyping. Total DNA was extracted from gastric biopsy specimens after digestion with proteinase K. Polymerase chain reaction that amplified the cagA gene and the s region and m region from the vacA gene was used with specific primers.

Biopsies were available for 67 subjects enrolled in the study. Among the remaining subjects, 1 (1.5%) was diagnosed with normal mucosa, 5 (7.5%) with superficial gastritis, 39 (57%) with antral diffuse gastritis, 15 (22%) with atrophic gastritis, and 7 (10%) with dysplasia. Only 35 biopsies had good DNA quality for *H. pylori* genotyping. 21 subjects (60%) were classified as infected with cagA-positive *H. pylori*, 8 (23%) as infected with cagA-negative *H. pylori*, and 6 (17%) as uninfected. Genotypic analysis for vacA gene showed different combinations: s1a/m1 (3), s1a/m2 (4), s1b/m1 (14), s1b/m2 (4), s1as1b/m1 (1), and s2/m2 (3). The presence of cagA was strongly associated with the vacA s1/m1 genotype (26) and atrophic gastritis (12). FONACIT G-2005000371.

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**Abstract no.: P07.19**

Quantification of Epigenetic and Genetic Second Hits in CDH1 During Hereditary Diffuse Gastric Cancer Syndrome Progression

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**Background:** Hereditary diffuse gastric cancer (HDGC) families carry CDH1 heterozygous germline mutations; their tumors acquire complete CDH1 inactivation through “second hit” mechanisms. Most frequently, this occurs via promoter hypermethylation (epigenetic modification), and less frequently via CDH1 mutations and loss of heterozygosity (LOH). We quantified the different second hits in CDH1 occurring in neoplastic lesions from HDGC patients.

**Methods:** Samples were collected from 16 primary tumors and 12 metastases from 17 patients among 15 HDGC families; CDH1 mutations, LOH, and promoter hypermethylation were analyzed. E-cadherin protein expression and localization were determined by immunohistochemistry.

**Results:** Somatic CDH1 epigenetic and genetic alterations were detected in lesions from 80% of HDGC families and in 75% of all lesions analyzed (21 of 28). Of the 28 neoplastic lesions analyzed, promoter hypermethylation was found in 32.1%, LOH in 25%, both alterations in 17.9%, and no alterations in 25%. Half of the CDH1 second hits in primary tumors were epigenetic modifications, whereas a significantly greater percentage of second hits in metastases were LOH (58.3%) (p = .0274). Different neoplastic lesions from the same patient frequently displayed distinct second hit mechanisms. Different second hit mechanisms were also detected in the same tumor sample.

**Conclusion:** The second hit in CDH1 frequently occurs via epigenetic changes in HDGC primary tumors and LOH in metastases. Because of the concomitance and heterogeneity of these alterations in neoplastic lesions and the plasticity of hypermethylated promoters during tumor initiation and progression, drugs targeting only epigenetic alterations might not be effective, particularly in patients with metastatic HDGC.
Abstract no.: P07.20
MUC1 Mucin-Mediated Signaling Pathways in Gastric Carcinoma Cells

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MUC1 mucin is a high molecular weight transmembrane glycoprotein that protects epithelial surfaces. MUC1’s highly conserved cytoplasmic domain (MUC1-CD) has been recently reported to be involved in cell signaling processes in different tumor models. MUC1-CD is phosphorylated by several kinases and participates in the modulation of multiple signaling cascades.

The aim of this work was to evaluate the impact of MUC1 expression in the phosphorylation status of cell cycle proteins and to identify MUC1 signaling partners in gastric carcinoma cells. The human gastric carcinoma cell line MKN45 was stably silenced (siRNA) for MUC1 and used to: 1, evaluate phosphorylation of cell cycle proteins by multi-immunoblots with phospho-site-specific antibodies (Kinexus) on total protein extracts, and 2, identify MUC1 signaling partners using coimmunoprecipitation and immunoblotting. MUC1-downregulated clones have shown a significantly increased phosphorylation in nucleophosmin (B23), CDK1/2, and ERK1/2 proteins. MUC1-CD binds ERK1/2 in these cells, suggesting that MUC1 mucin may be involved in direct modulation of Ras/Raf/MAPK signaling cascade in gastric cells. Furthermore, the relative levels of ERK1 and ERK2 expression vary between MUC1-downregulated clones and the scrambled control, suggesting that MUC1 may regulate not only the phosphorylation but also the transcription and/or stability of these proteins. The amounts of EGFR, Grb2, and B-Raf, that are molecules involved in Ras/Raf/MAPK signaling cascade, were shown to vary between MUC1-downregulated clones and the control. Further studies need to be performed to better elucidate MUC1’s involvement in Ras/Raf/MAPK pathway in gastric cancer cells.

Abstract no.: P07.21
Breastfeeding and H. pylori Infection in Pre-school Children

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Background: Helicobacter pylori infection is mainly acquired during childhood, it is recognized as a cause of gastritis and peptic ulcer and it has been classified as a group A carcinogen by World Health Organization. Childhood nutrition may influence acquisition of H. pylori infection. There are few current data to elucidate this association.

Aim: To investigate the relation between breastfeeding and H. pylori infection in children.

Methods: We interviewed, with questionnaire, 150 preschool children (98 male; mean age 5.9 years). The infection status of children and of the accompanying mother was determined by 13C-urea breath test.

Results: In all, 150 children and their mothers were included in the final analysis. H. pylori prevalence was 8.7% in children and 30.2% in their mothers. There was a strong association between children's and mother's infection. Of the children, 78.3% had ever been breastfed. Duration of breastfeeding showed a positive association with H. pylori prevalence in preschool age. Prevalence of H. pylori infection was higher in children breastfed compared to children who had never breastfed (9.8% vs 7.9%).

Conclusions: Even if breastfeeding is protective against a variety of infant illnesses and also has a beneficial effects on general morbidity, our study suggests no protective effect of breastfeeding history on children infection in preschool age.
Changes in mucin protein expression and in glycosylation are common features in preneoplastic lesions and cancer and are therefore used as cancer-associated markers. De novo expression of intestinal mucin MUC2 and cancer-associated sialyl-Tn antigen are frequently observed in intestinal metaplasia (IM) and gastric cancer. However, despite that these antigens often colocalize, MUC2 has not been demonstrated to be a carrier of sialyl-Tn. By using the in situ proximity ligation assay (in situ PLA) we herein could show that MUC2 is a major carrier of the sialyl-Tn antigen in all IM cases and in most gastric carcinoma cases. The requirement for in situ PLA for the presence of both antigens in close proximity increases the selectivity compared to measurement of colocalization, as determined by immunohistochemistry. The identification of which mucin is the carrier of a carbohydrate structure offers unique advantages for future development of more accurate diagnostic and prognostic markers.

Abstract no.: P07.24
**CDX2 Promoter Methylation is not Associated with mRNA Expression**

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Intestinal metaplasia (IM) of the stomach is a preneoplastic lesion defined by the transformation of normal gastric mucosa into an intestinal phenotype, driven by CDX2 expression. CDX2 is a homeobox transcription factor normally expressed in the intestine where it regulates the expression of several intestinal proteins. However, in certain pathologic conditions it is also aberrantly expressed in ectopic locations, such as in IM of the stomach. IM is most probably the end result of an adaptive response of the gastric tissue to *H. pylori* infection and subsequent chronic inflammation. Nonetheless, the underlying molecular mechanisms that dictate the alteration of a normal gastric differentiation program to an abnormal intestinal one are poorly understood. A small number of studies suggest that epigenetic regulation could have a relevant role in CDX2 expression, but none has clearly characterized the CDX2 promoter methylation status in IM. Therefore, our aim was to evaluate if methylation at the CDX2 promoter level could be a molecular mechanism responsible for its transcriptional regulation in the gastric context. The methylation status of CDX2 and its functional relation with mRNA expression was determined by the bisulﬁte-genome sequencing method in a panel of human gastric cancer cell lines and in specimens of the gastric mucosa, adjacent IM foci, and normal colonic mucosa. Our results show that gene-specific methylation does not constitute a primary mechanism regulating CDX2 expression both in gastric carcinoma cell lines and in gastric preneoplastic lesions since no consistent association was observed between methylation status and CDX2 expression.

Abstract no.: P07.26
**Association of Epstein–Barr Virus with Gastric Adenocarcinoma in a Population at High Risk of Gastric Cancer in Costa Rica**

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**Background:** Gastric adenocarcinoma is the second cause of cancer-related death globally. Although an association between gastric cancer (GC) and Epstein–Barr virus (EBV) has been established, the role of the virus in gastric pathogenesis is not well understood.
understood. GC associated with EBV is a nonendemic disease with worldwide distribution. The global number of cases is estimated at 50,000/year. The incidence rates (5–15%) vary geographically and an inverse correlation has been observed between the mortality from GC and the number of GC associated with EBV.

**Aim:** Costa Rica is one of the countries with the highest incidence and mortality rates in the world. The aim of this study is to determine the frequency of EBV associated GC in a population at high risk of GC and compare the clinical-pathological characteristics of GC associated and not associated with EBV.

**Methods:** Paraffin-embedded biopsies from 165 GC patients (92 males and 63 females) were analyzed by in situ hybridization for EBER1/2 as a marker for EBV infection.

**Results:** EBV was detected in 14 (8.5%) cases. EBV-associated GC was markedly more frequent in males (13%) than females (1.6%) as well as in proximal (22%) as compared to other anatomical locations (6%).

**Conclusions:** Although not significant with the small number of positive samples analyzed, EBV association tended to be more frequent in males and in patients with proximal GC. These data contribute to the characterization of the role of EBV in the etiology of GC in Costa Rica.

**Abstract no.: P07.28**

**H. pylori and Artificial Ulcer Healing After Endoscopic Submucosal Dissection for Early Gastric Cancer**

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**Backgrounds:** When endoscopic submucosal dissection (ESD) was introduced, artificial ulcer was bigger than conventional endoscopic mucosal resection. *Helicobacter pylori* was related with recurrence of peptic ulcer, not with healing.

**Aim:** To evaluate any relationship between *H. pylori* and ulcer healing.

**Patients/Methods:** From March 2003 to April 2008, 108 patients who received ESD for EGC were investigated retrospectively. All patients had urea breath test or rapid urease test. Follow-up endoscopy was performed around 50–90 days after ESD. They were divided into three groups according to ulcer type (scar type, ulcer stage 1, and ulcer stage 2) and evaluated to find factors related with delayed artificial ulcer healing.

**Results:** There were 108 patients (82 males, 26 females). Mean age was 61. Mean periods from procedure to follow-up endoscopy were 69 days. Fifty-nine cases were scar type, 18 cases were first stage of healing type, and 31 cases were second stage of healing type. There was no significant importance in existence of *H. pylori*, age, gross findings, fibrosis or ulcer, differentiation, invasiveness, resected size, and incomplete resection about artificial ulcer healing \((p > .05)\). Increasing age was related with delayed ulcer healing \((p < .05)\).

**Conclusions:** *H. pylori* may not be related with artificial ulcer healing but age be a predictable factor for healing rates of artificial ulcer.

**Abstract no.: P07.29**

**Prevalence of *H. pylori*-Negative Duodenal Ulcer (DU) Disease**

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**Background:** Recently, a relatively low prevalence of *Helicobacter pylori* infection in duodenal ulcer disease (DUD) has been described.

**Aim:** To review studies with low (<90%) prevalence of *H. pylori* infection in DUD.

**Methods:** We performed bibliographic searches in MEDLINE looking for the terms: (“*H. pylori*-negative” OR “non-*H. pylori*”) AND “ulcer”. Studies with low (arbitrarily defined as <90%) prevalence of infection were selected. Studies where all patients had comitant diseases or complicated DUD were excluded. The mean *H. pylori* prevalence was expressed as weighted mean to make due allowance for the number of patients included in each study.

**Results:** Fifty-six studies (including 12,096 patients) reported a prevalence of infection of <90%. Studies came from many different countries from several continents. Several studies included only children. The percentage of NSAID use in *H. pylori*-negative DUD patients was 21%, indicating that many of these studies did not exclude patients receiving these drugs. Mean *H. pylori* prevalence considering all studies with <90% *H. pylori* infection rate was 74%, but this figure increased up to 80% when NSAID use was excluded \((p < .001)\). The prevalence of infection slightly increased from 74% to 77% when only studies performing at least two diagnostic methods were included \((p < .001)\). Similarly, *H. pylori* prevalence was higher (80% vs 74%) when only studies obtaining biopsies from both antrum and corpus were included \((p < .001)\).

Finally, when all the possible explanations for *H. pylori*-negative DUD were excluded (NSAIDs, <2 diagnostic methods, only antral biopsies, previous PPIs or antibiotics, and gastrointestinal bleeding), the highest prevalence of *H. pylori* infection was obtained (90.5%).

**Conclusion:** The most common causes of *H. pylori*-negative DUD are the use of NSAIDs and the false negative results of diagnostic methods.

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P07 Gastric Cancer, Preneoplastic and Neoplastic Diseases, Pathology, and Pathophysiology

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Results: From 73 studies, including 16,080 patients, the mean \( H. pylori \) prevalence in patients with DU was 81.2% (95% confidence interval (CI): 80.6–81.8%). Nevertheless, the true incidence of \( H. pylori \)-negative DU disease remained difficult to determine because of the cross-sectional/retrospective nature of most studies. When only studies published from 1999 to 2003 were considered, the prevalence was 84%, and this figure was lower (77.2%) when a more recent study period (2004–2008) was considered (\( p < .001 \)). Prevalence of infection in DU disease in studies performed in Europe was higher than in those conducted in EEUU (83.9% vs 72.4%; \( p < .001 \)), while intermediate prevalences were described in other American countries. The highest rates of infection were reported in Japan (94.3%).

Conclusion: Mean prevalence of \( H. pylori \) infection in DU disease during the last 10 years was only 81%, and this figure was even lower (77%) when only the last 5 years were considered.

Abstract no.: P07.31
Clinical Evaluation and 5-Year Follow up of 673 Patients with Peptic Ulcers, Functional Dyspepsia, and Gastroesophageal Reflux Disease: Influence of \( H. pylori \) Infection and Successful Eradication

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Aim: We followed 673 patients with peptic ulcers (gastric ulcer – GU and duodenal ulcer – DU), nonulcer dyspepsia (NUD), and gastroesophageal reflux disease (GERD) and calculated the influence of \( Helicobacter pylori \) infection and eradication of this infection on clinical symptoms (epigastric pain, heartburn, nonspecific symptoms).

Patients: We followed 378 peptic ulcer patients (GU – 182, DU – 196), 173 with NUD and 122 with GERD of at least 5 years (endoscopy with four gastric biopsy, culture, and serology on every control). Frequencies of symptoms were calculated on the beginning and on the end of follow up.

Results: On first examination 79.7 GU, 85.2 DU, 72.3 NUD, and 69.7 GERD patients were \( H. pylori \) positive. The frequency of all symptoms was statistically equal among \( H. pylori \)-positive and \( H. pylori \)-negative patients. After 5 years statistically more ulcer patients (87.4% GU, 82.7% DU), then others (76.3% NUD and 74.6% GERD) were eradicated. Epigastric pain was less frequent in both ulcers and NUD patients regardless of eradication, but more frequent in both (eradicated and noneradicated) GERD patients. Heartburn was slightly more frequent in both ulcers and significantly in NUD patients, but less frequent in GERD patients, especially \( H. pylori \)-positive (71.0% and 81.3%) in comparison with 100% on first examination.

Conclusion: After 5 years, with one to four cycles of eradication therapy, statistically more ulcers than NUD and GERD patients were eradicated. Ulcers and NUD patients had less frequent epigastric pain, but GERD patients had pain more frequent, especially \( H. pylori \)-positive. Heartburn was slightly more frequent in both ulcers and significantly in NUD patient, but less frequent in GERD patients, especially \( H. pylori \)-positive.

Abstract no.: P07.32
The Effect of Prednisone on Gastric Corpus Atrophy: A Case Report

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Background: In the late 1950s several studies reported resolution of gastric atrophy and \( B_12 \) vitamin malabsorption after institution of steroid therapy. In 2006 Biondo et al. reported remission and gastric mucosal regeneration in experimental autoimmune gastritis in mice after prednisolone therapy.
Case Report: A 77-year-old male patient had anemia, B12 vitamin deficiency, and severe gastric corpus atrophy 20 years ago: pernicious anemia was diagnosed and since then he has had B12 vitamin injections every 3 months with resolution of anemia. In 2002 he had control gastroscopy for abdominal symptoms and persisting severe gastric corpus atrophy without dysplasia was revealed in histology. In the autumn 2008 he had weakness, pain and stiffness in shoulders and proximal muscles in limbs, and elevated erythrocyte sedimentation rate (ESR). Polymyalgia rheumatica was diagnosed, and prednisone therapy 20 mg daily was instituted in November 2008 with resolution of polymyalgic symptoms and drop in ESR and the prednisone dose was slowly tapered. However, he got mild dyspeptic symptoms and a new gastroscopy was performed in March 2009, with prednisone dose 12.5 mg at that time. Several biopsies from gastric corpus showed no changes in the grade of atrophic gastritis and no reinstitution of parietal cells.

Conclusion: Steroid therapy had no effect on persistent longstanding gastric corpus atrophy.

P08 Microbiology and Drug Resistance

Abstract no.: P08.01
Comparison of H. pylori in Silico Metabolic Model Predictions with Experimental Data

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The Systems Biology approach has been replacing the reductionist view that dominated biology research in the last decades. Present biochemical knowledge and genomic databases allowed the development of metabolic models for several organisms, which, however, are still incomplete. The availability of the genome sequence of Helicobacter pylori has allowed the construction of a genome-scale metabolic model for this organism. The purposes of this work were to study the growth of H. pylori in a chemically defined medium, to compare the experimental data obtained with the simulated data supplied by the model and analyze the composition of the in silico media used.

Cultures were grown at 37 °C under microaerophilic conditions in Ham’s F-12 medium supplemented with fetal bovine serum. Optical density and the counting of CFU/mL were performed for assessing the growth. OptiFlux, a software platform for metabolic engineering, which includes several tools such as flux balance analysis was employed for simulate the behavior of wild-type H. pylori under the conditions used in vivo.

The simultaneous use of both approaches allows to correct the in silico model, and on the other hand, to rationally adjust the medium components present in F-12. For instance pimelate, that has been considered to be essential in the latest metabolic model, is lacking in F-12 and is likely to be redundant in the model.

Our future work is not only to improve the genome-scale metabolic model, but also, identify potential targets for designing more effective drugs for the inactivation of H. pylori.

Abstract no.: P08.02
Prevalence of Primary, Secondary, and Tertiary H. pylori Resistance to Antibiotics in Algerian Patients

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Background: The efficacy of Helicobacter pylori eradication regimens is influenced by antibiotic susceptibility of strains. Data reporting antibiotic resistance after eradication treatment failure are lacking in developing countries.

Aim: In this study, performed between 2001 and 2009, we evaluated the evolution of antimicrobial resistant H. pylori strains isolated from adult naive Algerian patients and who had two unsuccessful treatments.

Methods: We tested 173 infected patients who had never received an eradication treatment (group 1), 66 patients who had failed a first eradication regimen (group 2), and 30 subjects who had failed a second retreatment (group 3). Susceptibility of isolates to four antibiotics, metronidazole (MET), clarithromycin (CLA), amoxicillin (AMO), and tetracycline (TET) was determined by E-test.

Results: Resistance to AMO and TET was not observed in the three groups. Rates of resistant strains in groups 1, 2, and 3 were respectively as follows: to MET : 37%, 64%, and 70% and to CLA : 12%, 23%, and 37%. Double resistance to MET and CLA was 5%, 23%, and 37%, respectively. Resistance to CLA was associated to MET resistance in 100% (26/26) of isolates.

Conclusion: Our results indicate that prevalence of H. pylori strains resistance to MET is high, and relatively high to CLA in Algerian-infected patients. Unsuccessful treatments significantly increase resistance to these antibiotics. First-line regimens more efficient than standard therapies are mandatory in areas with high primary antimicrobial resistance prevalence.
Case Report: A 77-year-old male patient had anemia, B12 vitamin deficiency, and severe gastric corpus atrophy 20 years ago: pernicious anemia was diagnosed and since then he has had B12 vitamin injections every 3 months with resolution of anemia. In 2002 he had control gastroscopy for abdominal symptoms and persisting severe gastric corpus atrophy without dysplasia was revealed in histology.

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Conclusion: Our results indicate that prevalence of \textit{H. pylori} strains resistance to MET is high, and relatively high to CLA in Algerian-infected patients. Unsuccessful treatments significantly increase resistance to these antibiotics. First-line regimens more efficient than standard therapies are mandatory in areas with high primary antimicrobial resistance prevalence.
Abstract no.: P08.03

Application of Blue Native Polyacrylamide Gel Electrophoresis for Analyzing H. pylori Outer Membrane Proteins

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Background/Aim: Blue Native polyacrylamide gel electrophoresis (BN-PAGE) has been first developed to analyze two dimensionally, the hydrophobic proteins such as mitochondrial membrane proteins. The aim of this study was to optimize and apply this technique for separation and analysis of Helicobacter pylori outer membrane proteins.

Method: Sarcosine insoluble outer membrane fractions of two different H. pylori clinical isolates were compared using different protocols after solubilization in dodecyl-beta-D-maltoside with detergent/protein ratio of 1/1 and detergent/dye ratio of 8/1. The result was applied to BN-PAGE in the first dimension and in SDS-PAGE in the second one. The gels were then stained by two methods of Coomassie-blue and silver staining.

Result: A good separation of the proteins was obtained, and fine differences were analyzable in comparison to normal one-dimensional SDS-PAGE.

Conclusion: Although the method of BN-PAGE should still be optimized for applying to prokaryotic outer membrane proteins, it would be an easy and nonexpensive method for analysis of H. pylori outer membrane proteins.

Abstract no.: P08.04

Evolution of H. pylori Susceptibility to Antibiotics During a 10-Year Period: Resistance to Clarithromycin is Related to Macrolides Consumption in Lithuania

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Aim: To evaluate changes in the prevalence of Helicobacter pylori strains with primary resistance to antibiotics during the last 10 years.

Methods: H. pylori susceptibilities were tested in 1998 (89 patients), 2001 (81 patients), and 2007/8 (90 patients). In 1998 and 2007/8 susceptibility to metronidazole (Mtz), clarithromycin (Cla), amoxicillin (Amx), and tetracycline (Tet) were tested by E-test and in 2001 by agar-dilution method. Susceptibility to ciprofloxacin (Cip) tested by E-test in 2007/8. Resistance breakpoints were: Mtz > 8 mg/L; Cla > 1 mg/L; Amx and Tet > 2 mg/L; and Cip > 1 mg/L.

Results: Two hundred and sixty strains were investigated. Resistance rates (1998, 2001, and 2007/2008) were: for Mtz: 24.7%, 33.3%, and 35.6%; for Cla: 1.1%, 3.7%, and 3.3%; and for Tet: 0%, 2.5%, and 0%, respectively. No cases of Amx resistance were detected. The resistance rate for Cip was 6%. Multidrug resistance observed in 7(2.6%) strains: four to Mtz and Cla; one to Mtz, Cla, and Cip; and two to Mtz and Cip. The total consumption of macrolides in 2007 was 1.8 DDD/1000 inhabitants/day, and use of clarithromycin constitutes 1.1 DDD/1000 inhabitants/day in Lithuania. Despite an increase of 1.5 times in the use of macrolides during 2003–2007, the total macrolide consumption remains low in Lithuania.

Conclusions: We have not observed significant changes in the susceptibility of H. pylori to the most widely used antibiotics during the recent 10 years. The low resistance rate to Cla might be related to the policy to avoid use of macrolides as first-line treatment for pulmonary and other infections.

Abstract no.: P08.05

Yeasts Inherit H. pylori Through Vertical Transmission of Their Vacuoles

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Introduction: Yeasts could be implicated in transmission of Helicobacter pylori through contact of mother to child or consumption of contaminated food and water. In this study we recruited the microscopy and polymerase chain reaction (PCR) to postulate whether yeast vacuole which divides during cell division could carry intracellular bacteria along with its contents to the new buds.

Materials and Methods: Thirteen oral Candida yeasts were examined for the presence of intracellular bacterium-like bodies (BLBs). Yeasts were subcultured >10 times on yeast extract glucose chloramphenicol agar. Yeast total DNAs were extracted and polymerase chain reaction (PCR) was performed for detection of jhp0947 gene at annealing temperature of 60 °C. Amplified products were examined by electrophoresis. H. pylori and Escherichia coli were used as controls. One jhp0947-positive yeast was stained by addition of Giemsa, Cresol red, or Brilliant cresyl blue dyes. Wet mounts were examined by light microscopy and photographs were recorded at time intervals.

Results: The size of PCR products (611 bp) was homologous to the one amplified from control H. pylori. The gene jhp0947 was detected in four of 13 yeasts. Microscopic observations revealed fast moving BLBs inside the vacuoles belonging to mother yeast and buds.

Discussion: H. pylori jhp0947 gene was amplified from the total DNA of Candida yeasts. Microscopic observations of yeast Candida revealed BLBs inside the vacuoles of mother cells and buds. Yeast vacuole which is a critical organelle for storage of phosphates and amino acids might provide an appropriate niche for H. pylori. This important organelle divides and partitions and takes with itself, its contents, including the intracellular bacterium.
Abstract no.: P08.06
How Efficient is Culture for H. pylori in Daily Clinical Practice?
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Background: Eradication failure of Helicobacter pylori is mainly due to increasing resistance to antibiotics. Culture is recommended before rescue treatment.

Aim of the Study: To determine the culture success rate in our hospital and to see if there is a difference between biopsies taken during routine endoscopy and biopsies taken after specific instructions to medical personnel.

Materials and Methods: Two biopsies were taken in antrum and corpus of 300 patients with an endoscopic suspicion of H. pylori infection for culture and for histopathology. A commercial transport medium was used and brought to the laboratory at the end of the day where it was cultured in three in-house agar plates. After 160 patients, medical personnel was informed about the importance of handling and transportation. The transport media were opened at the last minute, biopsies were put deeper into the transport media and were transported at noon to the laboratory.

Results: Culture was successful in 82.9%. In the first 160 patients, 74 patients were positive for H. pylori on histology. Culture failed in 25.7%. In the next 140 patients, 61 were positive for H. pylori on histology. Culture failed only in 6.6%. The difference is statistically significant (p = .007), using a chi-square test.

Conclusion: In our university hospital the global success rate of H. pylori culture is 82.9%. The culture success rate can be increased to over 90% if more attention is paid to handling and transportation of the culture media.

Abstract no.: P08.07
Anti-H. pylori Activity and Essential Oil Composition of Tanacetum turcomanicum from Iran
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Background: Helicobacter pylori is an important pathogen responsible for gastroduodenal diseases in humans. Antibiotic therapy and a combination of two or three drugs have been widely used to eradicate these infections. However, due to increased emergence of drug-resistant strains and adverse reactions to drugs currently administered, there is a need to develop anti-H. pylori agents with higher efficacy and less toxicity.

Methods: Anti-H. pylori activity of essential oil of Tanacetum turcomanicum, an endemic plant of Iran, was determined against clinical isolates and H. pylori ATCC 43504 using the disk susceptibility assay as well as measuring minimum inhibitory concentrations. The essential oil was isolated from the aerial part at flowering stage by hydrodistillation and analyzed by gas chromatography and gas chromatography-mass spectrometry.

Results: The essential oil showed anti-H. pylori activity by the disk sensitivity method and minimum inhibitory concentration. The main components of the essential oil were trans-chrysanthenyl acetate (19.2%), trans-thujone (13.5%), camphor (7.3%), α-pinene (2.9%), and thymol (2.2%).

Conclusion: As a result of the rise in antibiotic resistance, new sources of anti-H. pylori drugs are needed. The use of essential oils and identification of their chemical components may be of potential use in eradicating such problems.

Abstract no.: P08.08
Evolution of Resistance to Antibiotics in H. pylori, Algerian Clinical Isolates
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Background: To determine in vitro susceptibility to amoxicillin, tetracycline, clarithromycin, and metronidazole in Algerian Helicobacter pylori strains for six years.

Material and Methods: A total of 529 H. pylori strains were studied (267 adults, 189 pediatrics in the period 2001–2008 before treatment and 72 strains from adults after treatment).

Sensitivity to the four antibiotics was tested by E-test method (AB, Biodisk).

Resistance was defined as MIC for amoxicillin ≥ 1.5 mcg/mL; tetracycline ≥ 4 mcg/mL; clarithromycin ≥ 2 mcg/mL, and metronidazole ≥ 8 mcg/mL.

Results: All the strains tested showed MIC of < 1.5 mcg/mL to amoxicillin and MIC of < 4 mcg/mL to tetracycline; the prevalence of resistance to metronidazole and clarithromycin was similar in the isolates from treated and untreated ones: 46% and 44% for metronidazole; 9% and 6% for clarithromycin but is much higher for dual resistance to metronidazole and clarithromycin: 7% and 26% (p < .003).

In pediatric patients primary resistance is similar 42% vs 40% for metronidazole but it is increasing for clarithromycin, 6% to 15%.

Conclusion: They were no significant differences in primary resistance rates of H. pylori to antimicrobial agents between children and adults.

An important increase in the resistance to clarithromycin was observed in H. pylori strains throughout the period of study.

Abstract no.: P08.09
The Effect of 50 Hz Electromagnetic Field on H. pylori
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Aim: The changes in morphology and survival of Helicobacter pylori after exposure to 50 Hz electromagnetic field (EMF) 3 milliTesla for 20 and 120 minutes.
Methods: *H. pylori* NCTC11637 was cultured on Columbia blood agar with 7% defibrinated horse blood in anaerobic jar and Cook roasting bag at 37 °C for 4 days in microaerophilic condition. Spiral morphology was confirmed by modified Gram staining and wet mount slide prepared for motility. The bacterial suspension (∼10⁶CFU/mL) in Brucella broth was placed into an anaerobic jar and roasting bag then were exposed to 50 Hz EMF for 20 and 120 minutes in a microaerophilic condition at 25–28 °C. The controls of bacterial suspension were kept outside of the solenoid during exposure. Viability or culturability of 10⁴ and 10⁵ dilutions was determined after 20 and 120 minutes by spread plate colony count method. Each dilution from exposed and nonexposed samples was inoculated and incubated, and smears were stained by modified Gram staining.

Results: The number of *H. pylori* colonies and microscopic examination did not alter after exposure to EMF for 20 minutes in both; however, the recovery of *H. pylori* and colony counts were more reduced after 120 minutes in roasting bag than anaerobic jar according to control group. Some morphologic changes were observed from typical spiral form to elongated bacillary form. Therefore electromagnetic waves might be transmitted more easily through roasting bag. However, significant statistical difference was not found. Although the colonies in roasting bag were smaller than in anaerobic jar, typical spiral morphology was observed in both of them.

Conclusion: The exposure to 50 Hz EMF for 120 minutes had inhibitory effect on *H. pylori* colonies with reduction in bacterial viability as well as morphologic changes. The roasting bag was more convenient than anaerobic jar because of less risk of contamination.

Abstract no.: P08.10

**Epidemiology of Helicobacteriosis and Genetics of *H. pylori* Isolated in Tajikistan**

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The purpose of research was the analysis of an epidemiologic situation and studying of genetic characteristic of *Helicobacter pylori* isolated from people with gastritis and ulcer of stomach and duodenal gut in Tajikistan. The material from 1818 people has been investigated: from women – 1067 (58.6%) and from men – 751 (41.4%). Researches were spent by real-time polymerase chain reaction (PCR). An investigated material was stool of patients with gastrointestinal diseases and people without a pathology of these organs. *H. pylori* DNA was isolated from stool of 465 patients (25.5%). Frequency of detection at men and women was approximately identical (25.2% and 25.7% accordingly). Frequency of *H. pylori* detection at patients with gastritis, stomach ulcer, and duodenal ulcer was 26.9%. In persons without pathology of gastrointestinal tract this parameter was 20.0% that testifies about “healthy carrier”. It is necessary to note that our data will not be coordinated with research results from other countries, which inform about high (up to 90%) infectious rate by these bacteria. The PCR of chromosomal DNA has shown that distribution of genotypes *H. pylori* in the regions of Republic of Tajikistan has geographic specificity. There are combined ureB cagA cagH genotype of *H. pylori* more often meets in Dushanbe; genotypes vacS1 and vacA2 mainly present in Sugd area; cagH genotype is in Kulob region of Kharon area. Thus, this work testifies that helicobacteriosis has some epidemiologic features and specific genotypes of these pathogens circulate in various natural climatic and social regions of Tajikistan.

Abstract no.: P08.11

**Nationwide Survey of Antibiotic Resistance of *H. pylori* in Thailand**

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Objective: The aim of this study was to survey the antibiotic-resistant pattern of *Helicobacter pylori* infection in different geographical locations in Thailand during the year 2005–2009 and to compare the antibiotic-resistant strains among the patients with gastritis and peptic ulcer diseases.

Methods: A total of 2851 dyspeptic patients who underwent upper endoscopy from different regions (North, Northeastern, Central, and Southern) of Thailand during January 2005–May 2009 were enrolled in this study. Two antral gastric biopsies were obtained for culture, and susceptibility tests were performed using the E-test.

Results: A total of 853 patients (30%) were infected with *H. pylori* identified by rapid urease test. E-test for all four antibitiotics was successfully in 240 isolations (98 male, 142 female, mean age 53 years). The endoscopic findings demonstrated 178 gastritis patients, 56 peptic ulcer patients, and 6 gastric cancer patients. The prevalence of antibiotic-resistant *H. pylori* was: amoxycillin 6.5%, tetracyclin 3%, clarithromycin 3%, metronidazole 38%, and multidrugs 5%. Age, gender, and endoscopic findings were not statistically different between patients with resistant and sensitive strains.

Conclusion: *H. pylori* infection was common among Thai dyspeptic patients. The prevalence of metronidazole-resistant strain was high and remains the most common antibiotic-resistant strains in Thailand, whereas clarithromycin resistance has markedly declined in recent years. The reason for such a decline is likely due to the wide use of other newer antibiotics in place of clarithromycin.

Abstract no.: P08.12

**Trend of *H. pylori* Resistance to Antibiotics in Children Living in Vienna, Austria**

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Background: Increase of antibiotic resistance is a worldwide problem. At the turn of the millennium, approximately 25% of
Helicobacter pylori isolated from children living in Vienna, Austria, were resistant to clarithromycin and metronidazole. The intention of the present study was to examine retrospectively the development and current prevalence of antibiotic resistance of H. pylori infection in children.

Patients and Methods: Children having undergone upper endoscopy between March 2002 and March 2008 at two cooperating pediatric endoscopy units were included. H. pylori infection was diagnosed by rapid urease test, histology, and culture. If the latter was positive, susceptibility testing to amoxicillin, clarithromycin, and metronidazole by E-test followed. From March 2004 onwards, susceptibility to levofloxacin, tetracycline, and rifampin was additionally assessed.

Results: One hundred seventy-two of 897 children had a proven infection with H. pylori. The median age of infected children was 11.5 years (range 0.5–20.9 years). Antral mucosal nodularity was found in 65.1%, peptic ulcer disease in 5.8%, and intestinal metaplasia in 1.7% of infected children. Primary resistance to clarithromycin and metronidazole was 34.3% and 24.2%, respectively; double resistance was found in 10.3% of the strains. 0.6% was resistant to levofloxacin and tetracycline, respectively. No case of rifampin or amoxicillin resistance was detected.

Conclusion: In the last decade, the resistance of H. pylori to metronidazole remained stable. In contrast, the resistance to clarithromycin as well as the rate of double-resistant strains continued to rise.

Abstract no.: P08.13
European Multicenter Study on Antibiotic Susceptibility of H. pylori 2008–2009 – Data from the National Reference Centre for Helicobacter in Germany

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The National Reference Centre (NRC) for Helicobacter participates in the 3rd European In Vitro Antimicrobial Resistance Survey on H. pylori which was launched in May 2008. The aim of this study is to assess the primary resistance rates in clinical isolates against clarithromycin, levofloxacin, tetracycline, rifabutin, metronidazole, and amoxicillin in Europe. For susceptibility testing all centers use the same medium (Mueller Hinton), E-test strips, and protocol of E-test performance and reading. Additionally, the NRC performed real-time polymerase chain reaction (RT-PCR) from all gastric biopsies. Data are collected online via an electronic file.

The NRC investigated 393 gastric biopsies from previously untreated patients. A total of 180 biopsies were H. pylori negative (46%; culture and RT-PCR). In 100 biopsies RT-PCR was positive but culture negative (25%). MICs were determined for 113 isolates (29%). Primary resistance rates were 29% for metronidazole, 10% for clarithromycin, 15% for chinolones and 2% for rifabutin. None of the strains showed resistance to amoxicillin and tetracycline. Dual resistance (MZ, CLA) was present in 4% and triple resistance (MZ, CLA; QUI) in 1% of the isolates. RT-PCR of the 100 culture negative biopsies revealed wild type in 73% and resistance-mediating clarithromycin mutations in 27%, with A2143G mutation most common.

Even in naive patients resistant H. pylori were found up to 30%. These primary quotes are comparable to our ResiNet data except for clarithromycin, for which an increase from 6% (ResiNet) to 10% (multicenter) was observed. This study supports the increasing demand for susceptibility testing before eradication therapy.

Abstract no.: P08.14
Assessment of Clarithromycin Susceptibility in H. pylori Strains and Factors that may Predict Resistance

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Clarithromycin is an important antibiotic for Helicobacter pylori treatment that predicts eradication failure. We determined clarithromycin susceptibility and determine risk factors associated with resistance.

We studied H. pylori strains isolated from 118 patients, of whom 76.3% were born in Spain, 52.7% were children, 20.3% have been previously treated, and 66.1% were female. Clarithromycin resistance determined by E-test, strains was defined if MIC ≥ 1 mg/L. DNA extraction was carried out using the NucliSens easyMAG platform (BioMérieux). Sequences of clarithromycin-resistant and -sensitive strains were analyzed for mutations in the 23 rRNA gene. Vaca genotype and CagA status were determined by polymerase chain reaction.

Forty-two of 118 (35.6%) strains were resistant to clarithromycin by E-test. E-test results were confirmed for the presence of point mutation in 34 (88.1%) of these strains. However, eight H. pylori strains were resistant to clarithromycin by E-test but without point mutation in the 23 rRNA gene. Mutation A2143G was found in 85.3% of the strains, follows by A2142G (8.8%) and T2182C in 5.9% of the strains. Clarithromycin-resistant H. pylori strains were strongly associated with pediatric patients, with patients born in Spain and with patients previously treated (p ≤ .02). In addition H. pylori strains resistant to clarithromycin were more frequently vacA genotype s2/m2 and cagA negative than the susceptible strains (39.1% vs 11.2%, p value < .001).

Our results suggested that in Madrid, Spain, patients colonized with clarithromycin-resistant H. pylori are children, born in Spain, who have been treated, and are infected with a less virulent H. pylori strain.

Abstract no.: P08.15
Antibiotic Resistance of H. pylori Strains in the UAE and its Relation to CagA Gene: A Gene Associated to Gastric Cancer

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Helicobacter pylori has an extraordinary ability to establish infection in human stomach that can last for years. Its eradication remains
Abstract no.: P08.16
Retrospective Evaluation of H. pylori Clarithromycin Resistance in Children by Real-Time PCR

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Antibiotic resistance is a key factor in the failure of Helicobacter pylori eradication therapy. Resistance to clarithromycin is increasingly recognized as a major contributory factor in eradication failure. In this study our aim was to evaluate both H. pylori and its mutations that cause clarithromycin resistance among children over a 6-year period. For this purpose we studied the specimens from antrum and corpus of 483 pediatric patients. Biopsy specimens were stored in Tris-EDTA at −20 °C. Chromosomal DNA was extracted by cetyl-trimethyl-ammonium bromide method. After amplification of 96 bp region of 23S rRNA gene, clarithromycin resistance-related mutations of A2143C, A2143G/A2144G were analyzed by melting curve analysis on LightCycler Software version 3.5.3. Among 483 samples, 347 (71.8%) were positive by real-time polymerase chain reaction. Overall clarithromycin resistance was 31.4%. During 2003–2008 the resistance rates were 18.2%, 25.4%, 28.1%, 33.8%, 19.2%, and 60.9%, respectively. In 2008 clarithromycin resistance rates exhibited a distinct tendency to increase. The difference of clarithromycin resistance among age groups was not significant statistically. According to the Maastricht III Consensus Report, the threshold of clarithromycin resistance at which this antibiotic should not be used or susceptibility testing should be performed is 15–20%. As our resistance rate is 31.4% susceptibility testing must be performed even in the first-line treatment, especially in children < 12 years of age, for whom no rescue treatment is currently available.

Abstract no.: P08.17
The Primary Resistance for H. pylori in Brussels, Belgium

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Background: An increasing number of Helicobacter pylori-infected patients harbor antibiotic-resistant bacteria, resulting in treatment failure. An effective treatment should achieve an intention-to-treat eradication rate of over 80% and be based on known resistance patterns in the region.

Aim: To evaluate prospectively the primary antibiotic resistance of H. pylori in the area of Brussels, Belgium.

Patients and Methods: In every adult patient between February 2008 and April 2009 with an endoscopic suspicion of H. pylori, two biopsies were taken from the antrum and corpus for histology and culture. A questionnaire was filled in. Resistance for clarithromycin, metronidazole, and ciprofloxacin was tested.

Results: Three hundred and twenty-five patients were included. Culture was positive in 37.5%. Eighty of the 122 patients with positive cultures were eradicated before and were not used in the analyses. Resistance to amoxicillin was 0%, to clarithromycin (CL) 11.4%, to metronidazole (MET) 26.3%, and to ciprofloxacin (CIP) was 27.2%. Double resistance (MET/CL, MET/CIP or CL/CIP) was found in 17.5%, respectively 1.8%, 11.4%, and 4.4%. Triple resistance was found in 3.5%. Fifty-three percent showed resistance to at least one of the antibiotics stated above.

Conclusion: After decades of eradication therapy primary resistance to amoxicillin stays nearly absent. Primary resistance to clarithromycin was acceptable, allowing to continue the classical first-line therapy. Primary resistance to ciprofloxacin in the area of Brussels however increases rapidly, compared with results of 2005 (15.9%) and with the rest of Europe.

Abstract no.: P08.18
Antibacterial Activity of Plant Extracts Against H. pylori

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Introduction: Triple and quadruple therapies have been useful in eradication of Helicobacter pylori and diminishing of gastric disease symptoms. However, resistance to metronidazole is the main reason for failure in the current antimicrobial chemotherapies. Accordingly, search for novel drug candidates is warranted. Medicinal plants have always been a source of lead compounds.
for drug discovery. In this study, we evaluated the anti-\textit{H. pylori} activity of 25 medicinal plants on \textit{H. pylori}.


\textbf{Results:} Among the 25 plants, peel extracts of \textit{Punica granatum} exhibited a remarkable anti-\textit{H. pylori} activity. Peel extracts of nine types of \textit{Punica granatum} from different geographical regions of Iran exhibited a significant inhibitory effect. The size of inhibition zones (IZ) was 42 mm which was comparable to the medium size of IZD of metronidazole (MIC 8 µg/mL).

\textbf{Discussion:} Among the 25 plant extracts, the size of IZ of nine \textit{Punica granatum} indicated a significant inhibitory activity against \textit{H. pylori}. It is concluded that the peel extracts of most Persian pomegranate cultivars may contain compounds with anti-\textit{H. pylori} activity.

\textbf{Abstract no.: P08.19}

\textbf{Analysis of Metronidazole Resistance in \textit{H. pylori}: Identification of a Novel Factor of Antibiotic Resistance?}

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\textbf{Introduction:} Helicobacter pylori infection is eradicated with antimicrobial agents, such as metronidazole (MTZ). Mechanisms of MTZ resistance have been investigated; \textit{rdxA} and \textit{frxA} genes are considered major factors for resistance. The aim of this study was to characterize paired strains isolated from the same patient and to investigate the mechanism of MTZ resistance.

\textbf{Materials and Methods:} Paired strains, N1 and N1R, were isolated from the patient pre- and post-treatment failure. Antimicrobial susceptibilities were determined by E-test. The strains were characterized by arbitrarily primed polymerase chain reaction (AP-PCR). Virulence-factor genotypes were analyzed by PCR. Gene sequences were determined by PCR sequencing. Protein profiling was by 2D electrophoresis, and protein identification was determined by mass spectrometry.

\textbf{Results:} The N1 and N1R strains were, respectively, susceptible and resistant to MTZ. They had identical AP-PCR profiles and the same genotypes. Nucleotide mutations were present in \textit{frxA} but not in \textit{rdxA}; however, the nucleotide substitutions in \textit{frxA} are also in strains susceptible to MTZ. The 2D protein profiles were almost identical, but a few notable differences in protein abundance were apparent. N1R demonstrated a significant reduction in the relative abundance of one molecule compared to N1. The protein was identified by mass spectrometry analysis as a NAD(P)H-quinone reductase.

\textbf{Conclusions:} The MTZ-resistant isolate N1R arose due to exposure of the pathogen to MTZ in vivo. \textit{rdxA} was not associated with MTZ resistance in this strain. A potentially novel mechanism of MTZ resistance may have been identified using a proteomic approach and is currently being evaluated.

\textbf{Abstract no.: P08.20}

\textbf{Comparison of E-test, Real-Time PCR and Fluorescent In Situ Hybridization for the Detection of Clarithromycin Resistance in \textit{H. pylori}}

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Clarithromycin is a key component of most current triple-therapy regimens for treatment of \textit{H. pylori} infection. The most important point is that clarithromycin resistance is highly predictive of treatment failure of \textit{H. pylori}. The aim of our study was to detect clarithromycin resistance in \textit{H. pylori} by three methods.

Fifty-three \textit{H. pylori} samples were included in the study. E-test was performed on Mueller–Hinton agar medium +10% horse blood. Real-time polymerase chain reaction (RT-PCR) was done on Light Cycler. Chromosomal DNA was extracted by cetyl-trimethyl-ammomium bromide method. Melting peaks of 65 °C (± 2.0 °C) showed the detection of \textit{H. pylori} and the point mutation showing clarithromycin resistance was detected at 55 °C (± 2.0 °C). For fluorescent in situ hybridization (FISH) examination formalin-fixed paraffin-embedded gastric biopsies were sectioned. The sections were hybridized using the commercially available test system seaFast \textit{H. pylori} Combi-Kit, according to the manufacturer’s instructions.

From the 53 samples, 20 (%38) were resistant to clarithromycin by E-test, 27 (%51) by RT-PCR, and 24 (%45) by FISH.

In FISH and RT-PCR methods we are testing a biopsy sample, which may contain more than one strain but by E-test we probably are testing only one strain; which can explain the inconsistency of the results. For antibiotic susceptibility testing E-test is a very good method where the MIC results can be obtained, but in fastidious bacteria like \textit{H. pylori}, if only clarithromycin resistance is going to be tested according to our results RT-PCR and FISH are reliable methods.

\textbf{Abstract no.: P08.21}

\textbf{Trends in Primary Antimicrobial Resistance of \textit{H. pylori}}

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\textbf{Aim:} To study antimicrobial resistance of \textit{Helicobacter pylori} isolated from patients with no previous \textit{H. pylori} eradication therapy.
Patients and Methods: Cultures for H. pylori are taken from practically all patients who undergo gastroscopy at Herttoniemi Hospital and are suspected to have H. pylori infection. In this study, isolates from patients endoscoped during the years 2000 to 2008 and with no previous H. pylori eradication therapy were included.

Results:

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of isolates tested</th>
<th>Amoxicillin n (%)</th>
<th>Metronidazole n (%)</th>
<th>Clarithromycin n (%)</th>
<th>Tetracycline n (%)</th>
<th>Levofloxacin n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>21</td>
<td>12 (57)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2001</td>
<td>94</td>
<td>27 (29)</td>
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Conclusion: During the 9-year period, resistance to clarithromycin, the recommended first-line antimicrobial in eradication therapy, increased up to 17%. Constant surveillance of antimicrobial resistance is needed in order to be able to modify accordingly the guidelines for H. pylori eradication therapy.

Abstract no.: P08.22

Prevalence of Clarithromycin Resistance Mutations in H. pylori Isolated in Italy

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*Biologia Molecolare Ospedale di Pordenone, Pordenone, Italy; †Biodiversity S.p.A., Brescia, Italy; ‡Ospedale Tappeiner, Merano, Italy; §Istituto Superiore di Sanità, Roma, Italy; ¶Microbiologia e Virologia, Vivenza, Italy; **Microbiologia e Virologia, Vicenza, Italy

Aim: To assess the clarithromycin-resistant phenotype by cultural methods and point mutations associated by sequencing, and to assess the prevalence of different point mutations in Italian Helicobacter pylori strains.

Methods: Ninety-six strains of H. pylori isolated from patients living in different Italian areas have been studied. All strains were identified and tested for antimicrobial susceptibility. DNA was extracted using specific DNA extraction kits for Gram-negative bacteria. The DNA was amplified using specific primers for 23S rRNA gene region where the point mutations causing clarithromycin resistance are concentrated. The amplification products were purified and sequenced. Each sample was analyzed in duplicate in forward and reverse so as to detect the presence of point mutations.

Results: Thirty-nine of 96 (40.6%) strains were mutated. The mutations identified were A2143G (69.2%), A2142G (25.6%), and A2142C (5.1%). No other mutations were found. The correlation between the phenotypic expression of resistance or susceptibility to clarithromycin and the presence or absence of mutation was 90.6% (87 of 96 strains). For nine strains the results were discordant: four strains are phenotypically susceptible but resistance mutations are present; five strains are phenotypically resistant but no mutations are present. Further studies are underway to clarify the meaning of the discrepancies.

Conclusion: There is a good correlation between molecular and cultural test; however, it is necessary to further investigate the five strains resistant but without mutations on the region examined. The mutations A2142/3G and A2142C are the only ones present in 100% of our cases.

Abstract no.: P08.23

The Primary and Secondary Resistance of H. pylori Strains Isolated from Polish Children with Chronic Gastritis

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Background: Although a reduced prevalence of Helicobacter pylori infection has been observed recently the resistance of Helicobacter pylori strains to antibiotics used for eradication has been increased.

Aim: The aim of the study was to estimate the frequency of resistance among H. pylori strains isolated from children with primary and secondary H. pylori infection.

Materials and methods: Fifty clinical isolates were obtained in years 2007–2008 from patients aged 1.5–18 years with primary H. pylori infection, whereas 23 strains were isolated from children with reinfection. Antimicrobial susceptibility to six drugs
Aims and Background: Antibiotic resistance is thought to be more common in females but resistant patients use of metronidazole for gynecologic infection. Clarithromycin occurs in younger patients and females, probably reflecting greater resistance were measured on agar plate containing the antibiotics Metronidazole and Clarithromycin were obtained from charts, laboratory, and endoscopy reports. Frequencies of spontaneous metronidazole and clarithromycin resistance were measured on agar plate containing the antibiotics Metronidazole and Clarithromycin. Resistance to Metronidazole and Clarithromycin was detected in 32% (n = 16) of strains, whereas to Clarithromycin in 6% (n = 3). In group of patients with reinfection the resistance to both Metronidazole and Clarithromycin was detected in 48% (n = 11) strains, whereas to Metronidazole alone in 13% (n = 3) and Clarithromycin in 30% (n = 7).

Conclusions: The continuous surveillance to drug resistance among H. pylori in pediatric population is important. Resistance to Metronidazole and Clarithromycin increases with age. The high prevalence of multidrug resistance among children with primary infection is alarming.

Abstract no.: P08.24
Clinical Factors Associated with Clarithromycin and Metronidazole Resistance Among H. pylori Strains in an Irish Teaching Hospital

Adelaide and Meath Hospital, Tallaght, Ireland

Introduction: Helicobacter pylori infection is responsible for peptic ulcers and about 50% of all gastric cancer. Antibiotic resistance is rising and understanding this phenomenon is important in efforts to eradicate H. pylori.

Aims and Background: Antibiotic resistance is thought to be the single most important reason for treatment failures. The Maastricht-3 guidelines recommend tailoring antibiotic treatment to local resistance levels, therefore it is important to know resistance levels.

Method: Antimicrobial susceptibilities were tested by E-test. Frequencies of spontaneous metronidazole and clarithromycin resistance were measured on agar plate containing the antibiotics at concentrations of two and four times MIC value. Clinical data were obtained from charts, laboratory, and endoscopy reports.

Results: Two hundred and twenty-two patients were analysed, 98 were females. 31.5% had strains resistant to Metronidazole. 13.2% were noted to have strains resistant to Clarithromycin. 8.6% were noted to have strains resistant to both Metronidazole and Clarithromycin. Metronidazole resistance was more likely to occur in younger patients and females, probably reflecting greater use of Metronidazole for gynecologic infection. Clarithromycin resistance was more common in females but resistant patients tended to be older, which reflects widespread use for respiratory infections. Nonulcer dyspepsia was more common in both the Metronidazole- and the Clarithromycin-resistant groups than in groups that were sensitive to those antibiotics (65.2% vs 73.3% and 62.1% vs 72.1%, respectively). 23.2% and 41.4% respectively of the Metronidazole- and Clarithromycin-resistant strains were from patients who had had prior eradication compared to 14% and 13.2% of the corresponding sensitive strains.

Abstract no.: P08.25
High Frequency of Tetracycline Resistance in H. pylori isolates in Iran

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Introduction: Tetracycline is currently used for treatment of gastrointestinal disorders, including dyspeptic diseases due to Helicobacter pylori infection. Tetracycline resistance could lead to failure of antimicrobial therapies. In this study frequency of tetracycline resistance was assessed by disk diffusion method and the results were compared to previous studies.

Methods: Antral biopsies from 160 dyspeptic patients were cultured on selective Brucella blood agar under microaerobic conditions at 37 °C. Susceptibility of 160 isolates to tetracycline (MIC 0.5 μg/mL) was assessed. Since the rate of resistance was remarkably high, 50 of 160 strains were also tested at MICs 1 and 2 μg/mL.

Results: Among 160 isolates, 61 (38.1%) showed resistance to tetracycline (MIC 0.5 μg/mL). There was a remarkable increase in tetracycline rate compared to previous studies (MIC 0.5 μg/mL). Resistance frequency of 50 of 160 isolates which were further assessed with MICs 1 and 2 μg/mL was 17% and 10%, respectively.

Tetracycline resistance frequency of H. pylori isolates in three 3-year intervals (see Table 08.25).

Discussion: Compared to previous studies, the present study shows a significant increase in the frequency of tetracycline resistance. Increase in tetracycline resistance has been reported from Costa Rica (80%) and China (50%). Tetracycline has been used for prevention of infectious diseases and for growth promoting in chicken and cattle. Increase in tetracycline resistance of H. pylori could be due to common and large-scale use of this antibiotic in Iran which is an important public health warning.

Table 08.25

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Abstract no.: P08.26
In vitro Susceptibility of Antimalarials on H. pylori

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Dept. of Infection Control, Rigshospitalet, Copenhagen, Denmark

Introduction: The aim of this study was to investigate whether drugs used as treatment for malaria have any antibacterial effect on Helicobacter pylori in vitro. Drugs tested were chloroquine, mefloquine, quinine, artesunate, and artemether.

Background: There is a growing concern, regarding resistance towards antibiotics used in eradication of H. pylori. Therefore new drugs are needed in the treatment of the infection.

Materials and Methods: We used 36 strains of H. pylori, previously isolated from Lithuanian patients. Susceptibility testing was done with agar dilution method, using iso-sensitest agar supplemented with 10% horse blood. Concentrations ranged from 128 to 1 µg/mL, with twofold dilutions. Testing was done in duplicate. Minimal inhibitory concentration (MIC) was defined as the lowest concentration where there was no visible growth. MIC90 was defined as the lowest concentration where 90% of the strains had no visible growth.

Results: MIC90 for mefloquine was 64 µg/mL (MIC ranging 32–64 µg/mL). MIC90 for artesunate was 32 µg/mL (MIC ranging 14–32 µg/mL). MIC90 for chloroquine was 64 µg/mL (MIC ranging 32–128 µg/mL). The other drugs did not show any effect in concentrations tested.

Conclusion and Discussion: Mefloquine and artesunate had an antibacterial effect on H. pylori in vitro, but the MIC values were greater than for the antibiotics commonly used to treat H. pylori infection. Further studies are needed to see whether mefloquine and artesunate have an effect in vivo, and may be an adjunct in eradication of H. pylori.

Abstract no.: P08.27
Primary Resistance of H. pylori in Slovenia

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1Institute of Microbiology and Immunology, Ljubljana, Slovenia; 2ABAKUS MEDICO MEDICAL Diagnostic Center, Rogaška, Slovenia

Background: Antimicrobial resistance is the leading cause for treatment failure of Helicobacter pylori infection. The aim of our study was to asses primary resistance for key antibiotics used for eradication therapy and compare it with the secondary resistance.

Patients and Methods: Between 2007 and 2009 we isolated 97 strains of H. pylori from the naïve patients and 372 strains of H. pylori after multiple courses of failed eradication therapy. Antimicrobial susceptibility to clarithromycin, metronidazole, amoxicillin, ciprofloxacin, and tetracycline was assessed using phenotypical methods, E-tests, and breakpoint agar dilution method for metronidazole. We analyzed resistance profiles of the isolated bacteria.

Results: The primary antimicrobial resistance for metronidazole and clarithromycin was 18.6% and 17.5%, respectively. Combined resistance for both metronidazole and clarithromycin was 4.1%. We did not find any resistance against amoxicillin and tetracycline. 3.1% of isolates were ciprofloxacin resistant. On the other hand, the resistance to antibiotics was much higher in the case of secondary resistance.

Conclusions: Systematic surveillance of antimicrobial resistance of H. pylori is mandatory to adjust primary and subsequent eradication therapy, however such data in Slovenia is rare. In our study we detected high levels of primary clarithromycin and metronidazole resistance and very high level of secondary antimicrobial resistance to both key drugs. Our data conform to those published in the literature.

Abstract no.: P08.28
Detection of Clarithromycin Resistance-Mediated Mutations in the 23S Gene in H. pylori Strains from Lithuanian Patients

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1Department of Infection Control, Rigshospitalet, Copenhagen, Denmark; 2Institute of Microbiology, Kaunas University of Medicine, Kaunas, Lithuania; 3Department of Gastroenterology, Kaunas University of Medicine, Kaunas, Lithuania

Objectives: Clarithromycin resistance is the main reason for treatment failure of Helicobacter pylori infections. Eight clarithromycin-resistant H. pylori strains from Lithuanian patients were investigated for the presence of three important mutations involved in clarithromycin resistance.

Methods: Clarithromycin MICs were determined by E-test for a total of 88 H. pylori strains from Lithuanian patients (n = 40 from adults, n = 48 from children). Isolates showing a MIC value ≥ 1 µg/mL were further examined for the presence of A2146G, A2146C, and A2147G mutations in the 23S gene (GenBank accession number NC_000915) using the GenoType® HelicoDR test kit (Hain Lifescience GmbH). The experiment was performed according to the manufacturer protocol.

Results: Twenty percent (n = 8) of the H. pylori strains from children and 5% (n = 2) of the H. pylori strains from adults were clarithromycin resistant, and eight isolates (n = 7 from children, n = 1 from adults) were examined for clarithromycin resistance-mediated mutations in the 23S gene. Seventy-five percent (n = 6) had an A to G mutation at position 2147, and 25% (n = 2) had an A to G mutation at position 2146. 37.5% (n = 3) had a wild-type and a mutated version of the 23S gene, indicating the presence of multiple H. pylori strains in the culture sample.

Conclusion: All of the strains tested revealed mutations in the 23S gene found to be some of the most important mutations associated with clarithromycin resistance. The A2147G mutation was found to be the most widespread mutation among the H. pylori strains investigated.
P09 Clinical Trials and Novel Treatments, NSAIDs, COXIBs, ASA, and *H. pylori* Infection

**Abstract no.: P09.01**

**Levofloxacin in First-Line Triple and Sequential Regimens for *H. pylori* Eradication: Randomized Clinical Trial**


*San Pedro de Alcantara Hospital, Caceres, Spain; †La Princesa University Hospital and CIBEREHD, Madrid, Spain

**Background:** Eradication rates with standard triple therapy have declined to unacceptable levels. Levofloxacin-based and sequential regimens are novel promising first-line eradication therapies.

**Aim:** To compare standard triple therapy with other first-line alternatives: levofloxacin-based triple regimen, traditional sequential therapy (including clarithromycin), and levofloxacin-based sequential therapy (including levofloxacin instead of clarithromycin).

**Methods:** Two hundred and sixty-eight consecutive patients naive for eradication therapy were randomized into four therapeutic groups (67 patients in each group): 1, standard OCA: omeprazole, clarithromycin, and amoxicillin for 10 days; 2, triple OLA: omeprazole, levofloxacin, and amoxicillin for 10 days; 3, sequential OACM: omeprazole plus amoxicillin for 5 days, followed by omeprazole plus clarithromycin plus metronidazole for another 5 days; and 4, sequential OALM: omeprazole plus amoxicillin for 5 days, followed by omeprazole plus levofloxacin plus metronidazole for another 5 days. Eradication was confirmed by 

**Results:** Per-protocol cure rates were: OCA (66%; 95% CI: 54–77%), OLA (87%; 78–94%), OACM (85%; 76–93%), and OALM (90%; 82–97%). Intention-to-treat cure rates were: OCA (64%; 54–77%), OLA (84%; 74–92%), OACM (82%; 72–91%), and OALM (88%; 80–96%). Eradication rates were lower with OCA than with all the other regimens (*p* < .05). No differences in compliance or adverse effects were demonstrated among treatments.

**Conclusion:** In settings with high clarithromycin resistance, levofloxacin-based triple regimen, traditional sequential therapy (including clarithromycin), and levofloxacin-based sequential therapy (including levofloxacin instead of clarithromycin), are all more effective than standard triple therapy. Ten-day sequential levofloxacin treatment achieved the highest cure rate of approximately 90%.

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**Abstract no.: P09.02**

**Sequential Therapy for *Helicobacter pylori* Eradication: A Critical Review**

J. P. Gisbert,* X. Calvet,† J. P. A. O’Connor,‡ F. Megraud§ and C. A. O’Morain†

*La Princesa University Hospital and CIBEREHD, Madrid, Spain; †Eisai Co., Ltd., Tokyo, Japan; ‡Department of Gastroenterology, Faculty of Medicine, Oita University, Oita, Japan; §INSERM U853, Bordeaux, France

**Background:** Eradication rates with standard-triple therapy have declined to unacceptable levels. Sequential regimen is a novel promising therapy.

**Aim:** To critically review the role of sequential regimen for *Helicobacter pylori* infection.

**Methods:** Bibliographic searches in electronic databases, and manual search of abstracts from Congresses, were conducted up to May 2009.

**Results:** 1, Efficacy of sequential regimen: From the 25 studies included, most of them performed in Italy, and a total of 2482 patients, a mean *H. pylori* cure rate (intention-to-treat, weighted mean) of 91.3% was calculated [95% confidence interval (CI) = 90–92%]. 2, Comparison between sequential and standard–triple therapy: We updated previous meta-analyses including the 16 randomized controlled studies that, up to now, have compared these two regimens. Sequential regimen (1670 patients) was more effective than standard triple therapy (1704 patients): 91.6% (90–93%) vs 76.8% (75–79%) (intention-to-treat analysis). The odds ratio for this comparison was 3.1 (95% CI = 2.23–4.29). Results were heterogeneous. All studies published during 2008 and 2009 had lower than 90% eradication rates and, in some cases, ≤80%. Furthermore, most of the more recently published studies were unable to demonstrate differences between sequential and standard-triple therapy.

**Conclusion:** Sequential regimen is more effective than standard-triple therapy. So far, almost all the studies analyzing sequential therapy have been performed in Italy. Although, overall, mean eradication rate with sequential regimen is higher than 90%, a tendency towards lower efficacy with this regimen is observed in the more recent studies performed outside Italy. Therefore, the advantages of sequential treatment over standard-triple therapy should be confirmed in different countries and settings before a generalized change is recommended in first-line *H. pylori* treatment.

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**Abstract no.: P09.03**

**An Interim Report of a Large-Scale Nationwide Observational Study of *H. pylori* Eradication Rate in Japan**

T. Fujioka,* K. Sugizaki† and Y. Sakata†

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**Background and Aim:** The widespread use of clarithromycin in various treatments is considered to be associated with an
increasing prevalence of clarithromycin-resistant _Helicobacter pylori_, which causes the treatment failure in Japan. To elucidate the efficacy of first-line triple therapy using rabeprazole, amoxicillin, and clarithromycin, a large-scale nationwide observational study is ongoing.

**Methods:** This is an observational study involving 666 medical institutions and 807 doctors. Subjects who were diagnosed with gastric ulcer or duodenal ulcer and were confirmed to be _H. pylori_-positive received one of the two triple therapies: rabeprazole 10 mg + amoxicillin 750 mg + clarithromycin 200 mg or rabeprazole 10 mg + amoxicillin 750 mg + clarithromycin 400 mg, twice a day for 7 days. Subject enrollment and data collection were conducted through online electronic data capture system.

**Results:** A total of 2030 subjects were included in the efficacy analysis. The eradication rate in this population was 80.1%. The eradication rates based on other factors were: 1, a history of eradication: 80.9% in subjects without a history of eradication vs 51.0% in subjects with a history of eradication; 2, clarithromycin dose: 80.1% for 400 mg/day vs 80.0% for 500 mg/day.

**Discussion:** Despite the high prevalence of clarithromycin-resistant _H. pylori_ in recent years, the 80.1% eradication rate using rabeprazole-based regimens is considered excellent. The advantage of rabeprazole is to exhibit a rapid onset of increasing pH from the first day of administration, providing a quick and favorable pH environment for antimicrobial activities.

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**Abstract no.: P09.04**

**Second-Line Rescue Therapy with Levofloxacin After _H. pylori_ Treatment Failure. A Spanish Multicenter Study of 564 Patients**

**J. P. Gisbert** and On behalf of the _H. pylori_ Study Group of the Asociación Española de Gastroenterología (AEG)

La Princesa University Hospital, Madrid, Spain

**Aim:** Quadruple therapy is generally recommended as second-line therapy after _Helicobacter pylori_ eradication failure. However, this regimen requires the administration of four drugs with a complex scheme, is associated with a relatively high incidence of adverse effects, and bismuth salts are not available worldwide anymore. Our aim was to evaluate the efficacy and tolerability of a triple second-line levofloxacin-based regimen in patients with _H. pylori_ eradication failure, extending the experience of an ongoing multicenter study.

**Methods:** Design: Prospective multicenter study. Patients: in whom a first treatment with proton pump inhibitor–clarithromycin–amoxicillin had failed. Intervention: A second eradication regimen with levofloxacin (500 mg twice a day), amoxicillin (1 g twice a day), and omeprazole (20 mg twice a day) was prescribed for 10 days. Outcome: Eradication was confirmed with 13C-urea breath test 4–8 weeks after therapy. Compliance with therapy was determined from the interrogatory and the recovery of empty envelopes of medications. Incidence of adverse effects was evaluated by means of a specific questionnaire.

**Results:** A total of 564 consecutive patients were included. Mean age was 48 years, 45% were males, 37% had peptic ulcer, and 63% functional dyspepsia. Almost all (97%) patients took all the medications correctly. Per-protocol and intention-to-treat eradication rates were 76% [95% confidence interval (CI) = 73–80%] and 74% (70–78%). Adverse effects were reported in 20% of the patients, mainly including nausea (8%), metallic taste (5%), abdominal pain (3%), and myalgias (3%); none of them were severe.

**Conclusion:** Ten-day levofloxacin-based rescue therapy constitutes an encouraging second-line strategy, representing an alternative to quadruple therapy in patients with previous proton pump inhibitor–clarithromycin–amoxicillin failure, being simple and safe.

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**Abstract no.: P09.05**

**Fourth-Line Rescue Therapy in Patients with Three _H. pylori_ Eradication Failures**


†La Princesa University Hospital and CIBEREHD, Madrid, Spain; ‡Valme Hospital, Sevilla, Spain; ‡Costa del Sol Hospital, Malaga, Spain; §Galdakao Hospital, Vizcaya, Spain; ‡Donostia Hospital and CIBEREHD, San Sebastián, Spain; ∥Central de Asturias Hospital, Oviedo, Spain; ∥∥La Princesa University Hospital, Madrid, Spain

**Aim:** In some cases, _Helicobacter pylori_ infection persists even after three eradication treatments. Our aim was to evaluate the efficacy of an empirical fourth-line rescue regimen in patients with three eradication failures, extending the experience of an ongoing multicenter study.

**Methods:** Fifty-four patients in whom three eradication treatments had failed were prospectively studied. A first treatment with proton pump inhibitor (PPI)–clarithromycin–amoxicillin and a second with quadruple-therapy (PPI–bismuth–tetracycline–metronidazole) had been administered. In 13 patients, a third regimen with PPI–amoxicillin–rifabutin had been used, and therefore a fourth regimen with PPI–amoxicillin–levofloxacin (500 mg twice a day) was prescribed (10 days). In the remaining 41 patients, a third regimen with PPI–amoxicillin–levofloxacin had been used, and therefore a fourth regimen with PPI–amoxicillin–rifabutin (150 mg twice a day) was prescribed (10 days). Eradication was confirmed with 13C-urea breath test.

**Results:** Thirty-three patients received a fourth treatment with levofloxacin, and 41 with rifabutin. All patients but four (receiving rifabutin) completed the follow up. Compliance in the levofloxacin group was 100%, whereas four patients receiving rifabutin did not take correctly the medication (due to adverse effects: fever, myalgia, abdominal pain, and diarrhea (one patient) and vomiting (three patients). Incidence of adverse effects (none severe) was 39% with rifabutin (including leukopenia in two patients, and thrombopenia in another one), and 46% with levofloxacin (mainly myalgias). Per-protocol and intention-to-treat eradication rates with the fourth rescue treatment were, overall, 56% and 55%. Corresponding intention-to-treat cure rates for levofloxacin and rifabutin fourth line regimens were 69% and 49%.

**Conclusion:** Even after three previous _H. pylori_ eradication failures, an empirical fourth-line rescue treatment (with levofloxacin or rifabutin) may be effective in approximately 50% of the cases.
Abstract no.: P09.06

**H. pylori First-Line Treatment and Levofloxacin-Based Rescue Option in Patients Allergic to Penicillin**


1La Princesa University Hospital and CIBEREHD, Madrid, Spain; 2Costa del Sol Hospital, Malaga, Spain; 3Valme Hospital, Sevilla, Spain; 4Río Hortega Hospital, Valladolid, Spain; 5Central de Asturias Hospital, Oviedo, Spain; 6Donostia Hospital and CIBEREHD, San Sebastián, Spain

**Aim:** To assess the efficacy and tolerability of *Helicobacter pylori* first-line treatment (omeprazole–clarithromycin–metronidazole) and second-line rescue option (omeprazole–clarithromycin–levofloxacin) in patients allergic to penicillin.

**Methods:** Patients: Prospective multicenter study including consecutive patients allergic to penicillin. Therapy regimens: First-line treatment (62 patients): omeprazole (20 mg twice a day), clarithromycin (500 mg twice a day), and metronidazole (500 mg twice a day) for 7 days. Second-line treatment (29 therapy failures out of the aforementioned 62 patients): omeprazole (20 mg twice a day), clarithromycin (500 mg twice a day), and levofloxacin (500 mg twice a day) for 10 days. Outcome variable: negative 13C-urea breath test 8 weeks after completion of treatment.

**Results:** 1. First-line treatment (omeprazole–clarithromycin–metronidazole): Per-protocol and intention-to-treat eradication rates were both 55% [95% confidence interval (CI) = 37–63%]. Compliance with treatment and follow up was complete in 97% of cases (two patients were not compliant due to nausea). Adverse events were reported in eight patients (13%): seven nausea, one diarrhea. 2. Second-line treatment (omeprazole–clarithromycin–levofloxacin): Per-protocol and intention-to-treat eradication rates were both 76% (95% CI = 59–93%). Compliance with treatment and follow up was complete in all the cases. Adverse effects were reported in eight patients (28%), which did not prevent the completion of treatment: mild nausea/vomiting (seven patients), abdominal pain (one patient), diarrhea (one patient), and myalgias/arthritis (one patient).

**Conclusion:** In *H. pylori*-infected patients allergic to penicillin, the generally recommended first-line treatment with omeprazole, clarithromycin, and metronidazole has low efficacy for curing the infection. On the other hand, a levofloxacin-based regimen (together with omeprazole and clarithromycin) represents an encouraging second-line alternative in the presence of penicillin allergy.

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Abstract no.: P09.08

**Esomeprazole Versus “Old” Generation Proton Pump Inhibitors for the Eradication of *H. pylori*: A Meta-analysis**

A. G. McNicholl,* O. P. Nyssen,* X. Calver†† and J. P. Gisbert††

1Hospital Universitario de la Princesa, Madrid, Spain; 2CIBERehd, Spain, Spain; 3Hospital Sabadell, Barcelona, Spain

**Background:** Esomeprazole is a new generation proton pump inhibitor (PPI) but its efficacy in the eradication of *H. pylori*, compared with “old” PPIs, has not been well established.

**Objective:** To systematically review the efficacy of esomeprazole-based triple regimens, and to conduct a meta-analysis of studies comparing them with omeprazole-, lansoprazole-, or pantoprazole-based regimens in *H. pylori* eradication treatment.

**Methods:** Selection of studies: Randomized controlled trials comparing esomeprazole versus “old” generation PPIs (omeprazole, lansoprazole, or pantoprazole). Analysis was done using studies comparing regimens differing only on the PPI used, not on treatment’s duration or number of medication intakes per day. Search strategy: electronic and manual. Data synthesis: Meta-analysis combining the odds ratios (OR) (by intention-to-treat).

**Results:** The meta-analysis (including 10 studies, 1340 esomeprazole and 1562 “old” PPI-treated patients) showed better results for esomeprazole than for “old” PPIs (83.2% vs 77.6%; OR = 1.26; 95% CI = 1.04–1.53). Number needed to treat was 18. Subanalysis based on the PPI dose showed an eradication rate of 82.8% for esomeprazole 40 mg twice-a-day regimen, significantly higher than that for standard dose of “old” PPIs (71.8%; odds ratio = 1.54; 95% confidence interval = 1.10–2.16). The analysis of studies using 40 mg esomeprazole daily (low dose) showed no difference to standard dose of “old” PPIs. Results were homogeneous for all comparisons.
Conclusion: Esomeprazole 40 mg twice a day in triple therapy is slightly more effective than omeprazole, lansoprazole, and pantoprazole at standard doses for the eradication of *H. pylori*. However, cost-effectiveness of using high dose esomeprazole remains unclear.

Abstract no.: P09.09
Ten-Day Sequential Therapy Versus Standard Triple Therapy for *H. pylori* Eradication as First-Line Treatment in Korea

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Background: The eradication rate of proton pump inhibitor-based triple therapy for *Helicobacter pylori* infection is low because of resistance to clarithromycin or metronidazole. Recently, new strategy of sequential therapy has reported a good outcome. Aim: To assess whether a 10-day sequential therapy (ST) eradicates *H. pylori* better than standard triple therapy.

Methods: Sixty-nine patients (mean age 55.2 years, male 34, female 35) with proven *H. pylori* infection received ST (20 mg of rabeprazole, 500 mg of clarithromycin, and 1 g of amoxicillin, twice daily for the first 5 days, followed by 20 mg of rabeprazole, 500 mg of clarithromycin, and 500 mg of metronidazole, twice daily for the remaining 5 days). Sixty-nine individual matched controls were selected, who received triple therapy (40 mg of pantoprazole, 500 mg of clarithromycin, and 1 g of amoxicillin, twice daily for 7 days). Eradication was evaluated in 4 weeks with 13C-UBT.

Results: The eradication rate of ST was greater than that of triple therapy in per-protocol (92.8% (64 of 69) vs. 72.5% (50/69), *p* = .002). The study group consisted of 50 of gastritis, six of gastric ulcer, and 13 of duodenal ulcer (72.5%, 8.7%, 18.8%, respectively). The mild adverse effects were more frequent in ST than in triple therapy (27.5% vs 4.3%, *p* < .001). The most common side-effect was a bitter taste (11.6%) and the next was nausea and vomiting (5.8%) in ST, while nausea or diarrhea reported in triple therapy.

Conclusions: Compared to standard triple therapy, a 10-day ST is more effective on eradication of *H. pylori* infection as first line-treatment.

Abstract no.: P09.10
The Dual Therapy with Four Times Daily Dosing of Rabeprazole and Amoxicillin is Effective as the Third Rescue Regimen for Eradication of *H. pylori*

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Backgrounds/Aims: Most of patients who are refractory to usual standard therapies for *Helicobacter pylori* infection have rapid metabolizer genotype of CYP2C19 and are infected with strains resistant to some antimicrobial agents. However, most *H. pylori* strains are sensitive to amoxicillin. We tested whether dual therapy with the four times daily dosing of rabeprazole and amoxicillin is effective as the third rescue regimen for eradication of *H. pylori*.

Methods: Forty-nine patients who failed in eradication of *H. pylori* after two (first PPI/AMPC/CAM and second: PPI/AMPC/MNZ) or more regimens were enrolled to the study. They were treated with rabeprazole 10 mg four times a day and amoxicillin 500 mg for 2 weeks. At 4 weeks after treatment, they underwent the [13C]-urea breath test (UBT). When the result of [13C]-UBT was negative, they underwent the endoscopy and the successful eradication was confirmed by RUT.

Results: All patients completed the treatment. The eradication rate was 87.8% (43 of 49) (95% CI = 75.2–95.4%). No undesirable adverse events were observed during the study period.

Conclusions: The dual therapy with four times daily dosing of rabeprazole and amoxicillin is well tolerated and effective as the third rescue regimen for eradication of *H. pylori*.

Abstract no.: P09.11
Bismuth Improves the Effectiveness of *H. pylori* Eradication

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Hypothesis: Antibiotic resistance poses a serious problem for eradication of *Helicobacter pylori* via proton pump inhibitor (PPI) triple therapy. Addition of a different type of antimicrobial drug –tripotassium dicitrate bismuthate (TDB) might improve efficacy of conventional antimicrobial therapy.

Aims and Methods: In order to determine the ability of TDB to improve eradication of *H. pylori*, 60 patients (male 38, female 22, median age 40.1) with *H. pylori*-associated diseases (chronic gastritis, gastric, or duodenal ulcers) were observed. Upper gastrointestinal endoscopy was performed for all the patients. Polymerase chain reaction, microbiology, and urea test were used to detect presence of *H. pylori* before and after therapy in all observations. Patients were randomly assigned to three treatment groups with standard doses of drugs: first – a combination of omeprazole, amoxicillin, and TDB; second – omeprazole, amoxicillin, and josamycin; and third – omeprazole, amoxicillin, josamycin, and TDB.

Results: We found no clinical intergroup difference for symptom relief and endoscopy improvement (decrease of inflammation activity and duration of ulcers healing); however, microbiologically detected eradication rate was 79% in the first, 70% in the second, and 95% in the third group (*p* < .05).

Conclusion: We found that bismuth (BTD) increases the rate of eradication of *H. pylori*. The most effective regimen in our study was the third treatment regimen: bismuth-containing quadruple therapy.
Helicobacter pylori is the primary etiologic agent of peptic ulcer, duodenal ulcer, chronic gastritis, gastric adenocarcinoma, and related gastroduodenal disorders. Current triple therapies, including antibiotics and proton-pump inhibitors, have been successful, however adverse events, nonpatient compliance, and consequent relapse of H. pylori infections are common. Crude methanol extracts of Eucalyptus grandis stem bark were screened against a standard strain ATCC 43504 and 10 clinical strains of H. pylori using the agar diffusion method on Mueller–Hinton agar supplemented with defibrinated horse blood and grown in a microaerophilic cell surface hydrophobicity. The SAT titer decreased from 402. The urease activity of the three H. pylori strains tested decreased with increasing concentrations of the extract. The greatest inhibition of urease activity was observed in clinical strain UCH 97002 and UCH 98020 were inhibited by the extract to varying degrees. The minimum inhibitory concentration against the susceptible strains tested ranged from 0.39 and 1.56 µg/mL. The in vitro susceptibility of H. pylori to extracts of Eucalyptus camaldulensis and Eucalyptus torelliana

**Abstract no.: P09.13**

**In vitro Susceptibility of H. pylori to Extracts of Eucalyptus camaldulensis and Eucalyptus torelliana**

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The in vitro susceptibility of Helicobacter pylori to extracts of Eucalyptus camaldulensis and Eucalyptus torelliana (family Myrtaceae), Nigerian medicinal plants, was investigated in six strains of H. pylori, namely ATCC 4504, ATCC 47619, A2, T8984, 019A, and A6. The susceptibility of these strains was determined using a standardized agar dilution method (CLSI guidelines) with Mueller–Hinton agar, supplemented with defibrinated horse blood. The minimum inhibitory concentrations of the crude extracts against all the tested strains ranged from 12.5 to 400 µg/mL. Phytochemical screening of the plant extracts revealed the presence of tannins, saponins, and cardenolides. The anti-H. pylori activities demonstrated by these plants may be attributed to their chemical constituents, and explain their reported traditional uses, as well as their gastroprotective properties as demonstrated previously in experimental animals (Adeniyi et al., 2006). The result of this work suggests that in accordance with their traditional medical use in Nigeria, Eucalyptus camaldulensis and Eucalyptus torelliana have some therapeutic potential against H. pylori and thus, are of interest for the treatment of H. pylori infections.

**Abstract no.: P09.12**

**Anti-H. pylori Activities of Eucalyptus grandis (Myrtaceae): Effects on Susceptibility, Urease Activity, and Cell Surface Hydrophobicity**

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**Abstract no.: P09.14**

**Effectiveness of a 10-day Triple Therapy Combining Potent Acid Inhibition with Amoxicillin and Metronidazole for H. pylori Eradication in Clinical Practice. A Pilot Study**


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**Introduction:** Eradication rates of Helicobacter pylori infection with standard triple therapy are disappointingly low. New strategies of treatment are necessary. Previous studies have shown that potent acid inhibition using esomeprazole increases cure rates with triple therapy and that 10-day treatments are more effective than 7-day ones. In addition, the combination of amoxicillin plus metronidazole at full doses three times a day has shown to overcome metronidazole resistance and to achieve good eradication rates.

**Objective:** Assess the eradication rate of a new treatment regimen associating potent acid inhibition, amoxicillin, and metronidazole. Evaluate the tolerance to this regime.

**Material and Methods:** One hundred and thirty-six patients from eight different centres were included. H. pylori status was assessed before treatment by at least one of the following: histology, culture, rapid urease test, or urea breath test (UBT). Ten-day treatment, including esomeprazole 40 mg twice a day, amoxicillin 1 g three times a day and metronidazole 500 mg three times a day, was prescribed. H. pylori cure was assessed by UBT.

**Results:** A total of 136 patients were enrolled. Mean age was 52.6 ± 16 years and 59.6% were men. Indications for treatment were noninvestigated dyspepsia, functional dyspepsia, and gastric and duodenal ulcer. H. pylori eradication was achieved in 112 of the 127 patients who returned for follow up. Eradication rates were 82.4% [95% confidence interval (CI): 74.7–88.1] by intention-to-treat analysis and 88.2% [95% CI: 81.2–92.8] per protocol. The treatment was well tolerated.

**Conclusions:** The combination of esomeprazole, amoxicillin, and metronidazole is well tolerated and seems highly effective. This pilot study warrants the comparison with current standard treatments.
**Abstract no.: P09.15**

**H. pylori Eradication by Four Triple Therapies: Randomized Study in Double-Blind**


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**Aim:** To compare the eradication rates and tolerability of Four Triple Therapies administered in naïve patients.

**Methods:** It was a controlled, double-blind study: 238 patients (women: 70%, average age: 33.4 years, duodenal ulcer: 57%) infected by *Helicobacter pylori* were included. The infection was confirmed on the positivity of urea breath test (UBT) and/or two among the three following tests: histology, urease test, and culture. After randomization, patients received one of the four therapies: OAM7, OAM10, OAC, and RbcMT administered for 7 days with usual doses, with the exception of OAM10 prescribed for 10 days with a high dose of metronidazole (500 mg/3xj). Eight to 12 weeks after treatment, eradication has been affirmed on the negativity of UBT.

**Results:** The eradication rate of OAM7, OAM10, OAC, and RbcMT was in intention to treat (ITT) (and per protocol – PP) respectively 62.5% (67.3%), 74.2% (80.7%), 68.7% (79.3%), and 66% (68.6%), without a significant difference between the groups. The eradication rate of the resistant strains versus susceptible ones to metronidazole were respectively in ITT for OAM7, OAM10, and RbcMT: 62.5% vs 46.1%, 82.3% vs 66.7%, and 82.6% vs 50%. The difference was significant (p = .02) only for RbcMT. The success rate in resistant cases versus susceptible to clarithromycin (OAC) was 76.9% vs 16.7% (p = .01). Adverse events were minimal and more frequently with OAC. The compliance was complete in 98.4%.

**Conclusion:** The first-line tritherapies provide eradication rates of *H. pylori* not significantly different and are well tolerated. However, due to a slightly higher efficiency, low cost, and an increasing resistance rate to clarithromycin, the OAM10 regimen with a high dose of metronidazole seems more adapted in Algeria.

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**Abstract no.: P09.16**

**Original and Generic Pantoprazole-Based Standard Triple Therapies for the Eradication of *H. pylori* Infection in Duodenal Ulcer Patients**

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**Introduction:** Generic preparations are bioequivalent with original brands. However, it is not known whether their clinical efficiency is also similar.

**Aim:** To compare the original and two generic pantoprazole preparations1 in eradication of *H. pylori* infection in duodenal ulcer patients.

**Methods:** Seventy-six endoscopically (Fujinon EC250WL Video system) confirmed active duodenal ulcer patients were enrolled in an open, prospective, randomized study. *H. pylori* infection was confirmed from antrum and corpus samples by histology (modified Giemsa stain), immunohistology and, if needed, FISH. The patients were assigned to a 7-day standard regimen containing 2 × 40 mg pantoprazole, 2 × 1000 mg amoxicillin, and 2 × 500 mg clarithromycin; 24 cases received the original, while 26 and 26 patients, respectively, were given generic pantoprazoles (group G1 and G2). The eradication of the infection was controlled by 13C-urea breath test performed 6 weeks after treatment.

**Results:** The eradication rate on an intention-to-treat basis was of 70% (95% confidence interval, CI: 51.2–90.4%) in patients receiving the original pantoprazole, 73.9% (54.5–93.3) in group 1 (p = .63), and 77.8% (61.2–88.3) in group 2 (p = .57). Per-protocol rates of eradication were 73.9% (54.5–93.3) in the original group, 86.9% (72.0–99.8) in the G1 group (p = .26), and 84.0% (77.2–92.5) in G2 group (p = .39).

**Conclusions:** The clinical efficiency of the original and generic pantoprazoles in the eradication of *H. pylori* infection in duodenal ulcer is similar.

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**Abstract no.: P09.17**

**Role of Rebamipide in the Therapy of Peptic Ulcer Associated with *H. pylori***

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Infection with *Helicobacter pylori* results in the lesions of mucus barrier of stomach (MBS) and enhances the aggressive properties of gastric juice.

The aim was to improve the effectiveness of peptic duodenal ulcer (PDU) therapy by introducing rebamipide into the therapy complexes.

Forty-two PDU HP-associated patients were under the observation. The size of ulcerative lesions was 7.2 ± 2.1 mm. Mucus-producing function of MBS was evaluated by the content of N-acetylmuramic acid (NANA) and fucose in the blood serum and by their excretion with urine.

The patients were divided into two groups: I (n = 22) – pantoprazole–clarithromycin–amoxicillin – anti-*H. pylori* therapy 10 days; II (n = 22) additionally took rebamipide 300 mg/day for 28 days.

Clinical and endoscopic PDU remission in 28 days was recorded in 21 (95.5%) patients in group I and 22 (100%) patients in group II, while *H. pylori* eradication in 19 (86.4%) patients in group I and 21 (95.5%) patients in group II.

After the therapy had been completed the NANA concentration in group I reduced 1.15 times; group II 1.3 times (p < .001). NANA excretion with urine in group I decreased 1.1 times; group II 1.3 times (p < .05). Due to the anti-*H. pylori* therapy and rebamipide, the NANA excretion with urine was 1.2 times (p < .05) lower than that in group I. The blood serum concentration of fucose bound with proteins in group I patients increased 1.6 times (p < .001). The similar changes were detected when studying fucose excretion with urine.

The administration of rebamipide in combination with anti-*H. pylori* therapy increases the therapeutic effectiveness by enhancing duodenal mucous barrier resistance.
**Abstract no.: P09.18**

**Growth Inhibition of H. pylori by Sulfur-Containing Monoterpenes**

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It is well known that terpenes are the compounds used by plants in plant–plant, plant–insect, and plant–microbe interactions. Unification of terpene molecules and biogenic sulfur-containing groups into one molecule allows to obtain a new type of compounds with useful properties.  

**Aim:** To evaluate the antibacterial activity of monoterpenes and their sulfur-containing derivatives against Helicobacter pylori.  

**Materials and Methods:** Thirteen compounds (among them five initial terpenes, their sulfur-containing derivatives – six sulfides, one sulfoxide, and one sulfone) were tested. Growth inhibition was done on 20 strains H. pylori using a disk-diffusion method. Various disks were placed on brucella agar with 10% sheep blood earlier inoculated with 1 mL bacterial suspension in 0.9% NaCl (10^8–10^9 CFU/mL). Plates were incubated under microaerobic conditions at 37 °C for 3 days. Known antibiotics such as amoxicillin, clarithromycin, and tetracycline were used as a positive control for antimicrobial inhibition. The toxic effect of monoterpenes was tested on mice.  

**Results:** In our screening H. pylori isolates were more sensitive to the sulfide of methane series with the SCH₂COOCH₃ fragment. Sulfide of pinane structure with the CH₂S(CH₂)₂OH fragment and sulfide of pinane structure with the C₅SCH₂COOCH₃ unit have also antibacterial activity against H. pylori. The sulfide of methane series showed the minimal toxic effect on mice (oral acute toxicity with LD₅₀ 10,000 mg/kg).  

**Conclusion:** These findings suggest that sulfur-containing semisynthetic compounds obtained by chemical modification of naturally occuring monoterpenes extracted from the conifers (Pinus silvestris) have the inhibition activity against H. pylori and potential clinical application.

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**Abstract no.: P09.19**

**Antibiotic–Metal Complexes – An approach for Helicobacter Therapy**

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The present work incorporates the synthesis and characterization of metal complexes of antibiotics with metal ions like bismuth, zinc, copper, iron, and manganese active against Helicobacter pylori. Drugs that specifically inhibit H. pylori can be included in the category of antiulcer agents. It was decided to synthesize novel organometallic compounds of some antibiotics with metals. These complexes could behave as antibacterial as well as antiulcerative, a dual function, which is hitherto unknown in the therapy.

The selected antibiotics were reacted with metal salt in an alkaline medium to get the desired compounds. The selected antibacterial drugs in the present study are fluoroquinolones. These compounds were evaluated for antibacterial activity against some Gram-positive and Gram-negative microorganism. They were also evaluated for in vitro anti-H. pylori activity.  

From the experimental work performed following conclusions were drawn,  

1. These compounds were characterized using various spectral analysis (UV, IR, Mass), thermal analysis (DSC, TGA), and chemical analysis (Elemental composition and Karl–Fisher aquametry).  

2. Preliminary microbiologic screening of the synthesized compounds showed excellent results.  

3. In vitro anti-H. pylori activity was performed on 19 different strains of H. pylori. It was observed that the MIC values for the MFCs were less than the corresponding ligands. The results were statistically analyzed by Tukey’s multiple comparison test (p values < .05).  

4. Some of these complexes also showed promising activity against antibiotic-resistant strains of H. pylori.

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**Abstract no.: P09.20**

**Levofloxacin and Bismuth in H. pylori Rescue Therapy**

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**Objective:** More efficient rescue therapies are needed after Helicobacter pylori eradication failures.  

**Patients:** Forty-one consecutive patients, (32 women, median age 50 years, range 22–68) with either H. pylori resistant to both metronidazole and clarithromycin (11 patients) or failure after several courses including at least one clarithromycin-based course were included. Prior to this one, the patients had received a mean 2.7 (range 0–9) eradication courses. Indication for eradication was peptic ulcer in seven patients and dyspepsia in 34. The patients received a 7-day (45 patients) or 10- to 14-day (7 patients) course of levofloxacin 500 mg twice a day, ranitidine bismuth citrate 400 mg twice, a double dose of proton pump inhibitor (esomeprazole 40 mg twice a day or lanosprazole 30 mg twice a day), and amoxicillin 500 mg four times a day for those not allergic to penicillin, and tetracyclin or metronidazole for those with penicillin allergy (10 patients).  

**Results:** One patient stopped the course on the first day, and another never did the follow-up tests.  

Of the 39, five showed a positive urea breath test. In 34 of 39 (87%), the successful eradication was confirmed by both breath test and serology in 19, by breath test alone in 13, by serology in one and by stool antigen test in one. Three patients had a sick leave of 3–7 days during the antibiotic course. One of these also reported tendinitis-like symptoms.  

**Conclusions:** Our results suggest that bismuth might be of value in rescue therapies even in combination with levofloxacin, and this combination should be studied along other rescue therapies.

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**Abstract no.: P09.21**  
**Back to the Future: New Studies on Dual PPI Plus Amoxicillin Anti-*H. pylori* Therapy**  
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Background: Cure rates of empiric triple therapy are now below 80%. Studies with CYP2C19 slow metabolizers have shown that proton pump inhibitor (PPI) plus amoxicillin dual therapy can reliably cure >90%. The dose of PPI that will provide equivalent results with fast and slow metabolizers is unknown.  
Aim: Test high dose PPI plus amoxicillin given every 8 hours for *H. pylori* eradication.  
Methods: *H. pylori*-infected individuals received esomeprazole 40 mg plus amoxicillin 750 mg every 8 hours for 14 days. The protocol was planned based on the “efficient identification strategy” (Clin Gastroenterol Hepatology 2009;7:145) with stop criteria of six or more failures within 50 patients or a cure rate of <80% after 30 or more patients.  
Results: Thirty-six patients were entered (five women, 31 men; average age 58). Twenty-six were cured. Intention to treat (ITT) = 72.2% (95% confidence interval (CI) = 56–84%) and per protocol = 74.2%; (95% CI = 56–87%) or a grade F result. There were no significant side-effects. Compliance was 85% or greater in all (100% in 91.6%). All treatment failures received triple (if susceptible to clarithromycin) or concomitant therapy.  
Conclusions: The dose or frequency of PPI administration chosen for this study was insufficient to consistently achieve the objective of providing >90% *H. pylori* eradication. In successful Japanese studies, the PPI has typically been administered every 6 hours. Subsequent studies will evaluate long-acting PPIs, more frequent dosing of standard PPIs, or concomitant administration of sodium bicarbonate. Dual therapy with the doses tested here is at least as successful as current empiric triple therapy.

**Abstract no.: P09.22**  
**The Effect of Monocast Red Wine and Fruit Juices on In Vitro Growth of *H. pylori***  
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Helicobacter pylori infection is considered the human chronic infection, most prevalent in the world. In developing countries, *H. pylori* affects 80% to 90% of the population and in developed countries the prevalence is between 25–50%. It is associated to many clinical situations, like chronic gastritis, ulcer (gastric and duodenal), and gastric cancer.  
The therapy, using different combinations of antibiotics has been extensively used, in the eradication of *H. pylori*. Alternatives are needed due to the developing resistance by the bacteria as well as the secondary effects of this therapy.  
The aim of this study was to evaluate, in vitro, the inhibitory effect of different drinks: monocast red wine and natural fruit juices from grape, pomegranate, pineapple, grapefruit, orange, and lemon in *H. pylori* growth.  
Nineteen isolates from the collection of Faculdade Engenharia Recursos Naturais – Algarve’s University were studied. The isolates were selected in accordance to their virulence factors. The reference strain (Cag+ s1/m1) was a CCUG 15818 from the Culture Collection of Gothenburg’s University. These isolates have been inoculated in Columbia agar, supplemented with 10% blood and different concentrations of the agents to be tested. Incoculated plates were incubated during 48 hours at 36 ± 1°C in microaerophilic conditions.  
The tested agents showed ability to control *H. pylori* growth for all the tested strains. Concentrations of wine ≥ 22.5% showed growth inhibition. The natural fruit juices presented variable degrees of inhibition.  
The results showed that the studied agents could be an alternative to the eradication of *H. pylori* infection.

**Abstract no.: P09.23**  
**Eradication of *H. pylori* may be with 30% Water Extract of Propolis**  
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Authors determined sensitivity of Helicobacter pylori to propolis in the laboratory conditions. The work was carried out on three strains of *H. pylori*: two laboratory strains and one clinical isolate from a patient with chronic gastritis. The obtained findings showed that at the concentration of the dry substance of propolis in the culture medium amounting to 0.12–0.14%, no growth of any strains of *H. pylori* was observed. We thus came to a conclusion that propolis may safely be used as an agent possessing an antibacterial activity.  
The patients with a duodenal ulcer and the chronic gastritis, associated with *H. pylori* was exposed antibacterial therapy. The patients received 30% water extract of propolis in quantity 100 mL twice per day during 2 weeks. It is surveyed in 44 patients. The received results have defined a level of eradication at 63.6%. Thus eradication came in case of a low and an average degree semination of *H. pylori*, a mucous membrane of a stomach. In the other 36.4% of cases, decreased level of semination of *H. pylori* with high up to low was marked.  
Thus, 30% water extract of propolis may be use for eradication of *H. pylori*.

**Abstract no.: P09.24**  
**Prevalence of *H. pylori* in Perforated Peptic Ulcer Patients Over a 5-year Follow-up**  
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Aim: The aim of the study was to assess the persistence of Helicobacter pylori in the mucosa of antrum of perforated peptic...
ulcer (PPU) patients over a 5-year follow up using molecular methods and detect its association with initial surgical and antibacterial treatment.

**Patients and Methods:** 43 patients were operated for PPU using either definite methods – truncal vagotomy (Def; 19 patients) with traditional triple therapy without omeperazole or nondefinite methods – excision or suturation of ulcer (Non-def: 24 patients) with triple therapy during 7 days and followed up over 5 years. DNA of *H. pylori* was isolated from gastric antrum mucosa and the polymerase chain reactions were performed to detect the *ureA*, *nagK*, and *vacA* alleles.

**Results:** In a 5-year follow up, *H. pylori* was found in 32 of 43 (74%) patients, whereas only in four patients the *H. pylori* strains with lower virulence emerged (change from s1am1 to s1am2). The eradication was achieved after 2–4 months in six, 1 year in one, and 5 years in four patients while it did not depend on the treatment methods (by Def in 11% and non-def in 38% of patients, \( p = 0.77 \)).

**Conclusion:** In patients with PPU the high persistence of *H. pylori* strains after treatment and low recurrence rate after eradication in a 5-year follow up were detected. No association with different treatment methods and the outcome of the eradication therapy was found.

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**Abstract no.: P09.25**

**Anti-Helicobacter Activity and Gastroprotective Effect of the Mixture of Alpha and Beta Amyrin from Protium heptaphyllum**

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Treatment failure is a major cause of concern for the *Helicobacter pylori*-related gastroduodenal diseases. Many plant-derived triterpenoids have been shown to produce gastroprotective effects in experimental and clinical studies. *Protium heptaphyllum* March (Burseraceae), popularly known as almecega grows abundantly in the Amazon region and in various other parts of Brazil. The resinous exudate collected from the trunk wood of this plant in its natural form is a reputed folk remedy with anti-inflammatory, analgesic, expectorant, and wound-healing actions. Phytochemical studies on the resin revealed the presence of several monoterpenes and some pentacyclic triterpenes that include a mixture of alpha and beta amyrin. Recently, it was reported that the crude resin from *P. heptaphyllum* and the mixture of amyrin had a gastroprotective property against ethanol-induced gastric damage in mice. In this study we examined the in vitro antibacterial activity of resin, essential oil, and amyrin mixture from *P. heptaphyllum* against 26 clinical isolates and reference strains of *H. pylori*, and in vivo activity and gastroprotective property induced by *H. pylori* infection. The MIC and MBC of resin and amyrin mixture ranges from 50 µg/mL to 160 µg/mL, and CBM results from acid microdilution assay (pH 4.0) showed similar values. The antimicrobial effect of amyrin mixture in *H. pylori*-infected C57BL/6 mice and its efficacy in reducing the gastric damage due to infection were examined. Amryn mixture, surprisingly, did not show therapeutic potential against *H. pylori* infection in this in vivo assay, but was highly effective in restoration of *H. pylori*-induced gastric damage.

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**Abstract no.: P09.26**

**Possibility of Levofloxacin-Based Triple Therapy for *H. pylori* Eradication Among Inhabitants of Eastern Siberia**

V. V. Tsukanov, O. S. Rzhavicheva, E. Y. Kupershtein, V. N. Sharypova and J. L. Tonkikh

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**Aim:** To study the efficacy of levofloxacin based triple therapy for *Helicobacter pylori* eradication.

**Methods:** Research was executed in 36 patients with duodenal ulcer (22 men, 14 women) in two large clinics of Krasnoyarsk. For *H. pylori* eradication the 7-day therapy was applied: omeprazole (20 mg twice a day), amoxicillin (1 g twice a day), and levofloxacin (250 mg twice a day). *H. pylori* was diagnosed by histologic and urease in all patients in the beginning of treatment and in one month after treatment. For clinical dynamics assessment questionnaires were used, which allowed to estimate symptomatology and side-effects daily. Endoscopic control was carried out in 21 days after the beginning of treatment.

**Results:** Eradication rates by intention-to-treat analysis was 72.2% and the eradication rates by per-protocol analysis was 74.3%. Dynamics of ulcerative defects healing was satisfactory. In 3 weeks from the beginning of therapy ulcers were cicatrized in 35 of 36 patients. Due to the side-effects eradication was cancelled in one patient. Other patients tolerated therapy well, with the minimal frequency of side-effects.

**Conclusion:** Levofloxacin-based triple therapy has not allowed to achieve necessary level of efficacy.

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**Abstract no.: P09.27**

**The Effectiveness of Anti-Helicobacter Pharmacotherapy with Proton Pump Inhibitor, Bismuth Subcitrate, Clarithromycin, and Amoxicillin in Patients Suffering from Gastroesophageal Reflux Disease**

I. Palii and S. Zaika

The Pirogov Vinnytsia National Medical University, Vinnytsia, Ukraine

The necessity of *Helicobacter pylori* eradication in patients suffering from gastroesophageal reflux disease is proven. Rapid development of *H. pylori* resistance to the main components of anti-*H. pylori* eradication schemes working out. **Aim:** To study the effectiveness of the *H. pylori* eradication scheme (proton pump inhibitor, bismuth subcitrate, clarithromycin, amoxicillin) as a second-line treatment variant in patients suffering from gastroesophageal reflux disease (GERD) with previously failed anti-*H. pylori* pharmacotherapy.
We examined 15 *H. pylori*-infected patients with GERD (group I) who have never received anti-*H. pylori* pharmacotherapy and 15 *H. pylori*-infected patients with GERD (group II) with previously failed anti-*H. pylori* pharmacotherapy.

Lansoprazole, clarithromycin, and amoxicillin were prescribed to group I patients. Lansoprazole, bismuth subcitrate, clarithromycin, and amoxicillin were prescribed to group II patients. The treatment duration was 7 days. *H. pylori* eradication control by means of 13C-urea breath test was conducted in 4 weeks after the treatment.

**Results:** *H. pylori* eradication was achieved in 86.7% of the group I patients. Successful *H. pylori* eradication was confirmed in 93.3% of the group II patients (*p > .05*).

**Conclusions:** The administration of the first-line anti-*H. pylori* pharmacotherapy scheme (lansoprazole + clarithromycin + amoxicillin) is appropriate to the patients with GERD for primary *H. pylori* eradication. Such treatment scheme results in *H. pylori* eradication in 86.7% of the cases.

Second-line *H. pylori* eradication scheme (lansoprazole + bismuth subcitrate + clarithromycin + amoxicillin) has a high degree anti-*H. pylori* efficiency (93.3%) in patients suffering from GERD with previously failed anti-*H. pylori* pharmacotherapy.

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**Abstract no.: P09.28**

**Viability of Helicobacter-Like Organisms After 21 Days of Treatment with Doxycycline in Asymptomatic Dogs: A Preliminary Study**

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Doxycycline belongs to the tetracycline group of drugs to treat bacterial infections, including pneumonia, of skin, genital, and *Ehrlichia canis* and *Ehrlichia platys*. The objective of this study was to evaluate the viability of *Helicobacter*-like organisms after 21 days of treatment with doxycycline in asymptomatic dogs. Seven dogs were treated for 21 days with commercial doxycycline at a dose of 20 mg/kg/day. Stomach samples were collected by gastroduodenoscopy, before and after doxycycline treatment. Gastric tissue sections were prepared. Additionally, it also used a special staining (Warthin–Starry, WS). None of these dogs showed previous clinical signs of gastrointestinal disease. In the gastroduodenoscopy study after doxycycline treatment, we found a normal gastric mucosa in one of seven dogs, acute superficial gastritis in three of seven, chronic superficial gastritis in one of seven, chronic atrophic gastritis one of seven, and chronic ulcer-erotic gastritis in two of seven. Histopathologic study showed acute surface gastritis, focal erosion, and hyperkeratosis with lymphocytes infiltration in the lamina propria. All samples were positive for Warthin–Starry staining. The presence of spirochetal shaped bacteria in the gastric mucus and fundus mucous glands were observed to be associated with gastric lesions. Gastroduodenoscopy after treatment with doxycycline revealed: four of seven gastric mucosal normal, two of seven superficial acute gastritis, and one of seven superficial chronic gastritis. Histopathology showed acute gastritis surface, erosion focal, and hyperkeratosis focal infiltrated lymphocytes in the lamina propria. None of the samples showed spiral-shape bacterial in all Warthin–Starry stainings which were negative after the treatment with doxycycline. We conclude that doxycycline is effective for *Helicobacter*-like organism viability in the gastric mucosa of dogs.

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**Abstract no.: P09.29**

**The Correction of Large Bowel Microflora in *H. pylori*-Infected Patients Suffering from Gastroesophageal Reflux Disease**

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The therapeutical schemes recommended by international agreements for eradication of *Helicobacter pylori* do not result in the restoration of the large bowel microflora. Moreover, in some cases they can even promote saprophyte microflora growth inhibition, not influencing the pathogenic microflora.

**Aim:** To study the large bowel microflora status in patients with gastroesophageal reflux disease (GERD) after anti-*H. pylori* pharmacotherapy combined with the remedy containing *Saccharomyces boulardii*. We examined 25 *H. pylori*-infected patients with GERD. Lansoprazole, clarithromycin, and amoxicillin were used as anti-*H. pylori* therapy. The remedy containing *Saccharomyces boulardii* was prescribed from the first day of the treatment according to the following scheme: two capsules twice a day during 3 days, then one capsule twice a day for 7 days.

**Results:** Applied anti-*H. pylori* pharmacotherapy scheme results in the normalization of the large bowel microflora in 76% of the patients with GERD. Anti-*H. pylori* pharmacotherapy combined with *Saccharomyces boulardii* does not influence (*p > .05*) the large bowel saprophyte microflora (lacto- and bifidobacteria) and results in a significant decrease of the weak enzymatic *E. coli* and hemolyzing *E. coli* amount (*p < .001*). At the same time the *H. pylori* eradication was achieved in 92% of the patients.

**Conclusion:** The addition of *Saccharomyces boulardii* to the anti-*H. pylori* pharmacotherapy results in the restoration of the qualitative and quantitative composition of large bowel microflora in 76% of the patients with GERD and does not cause the decrease of the *H. pylori* eradication effectiveness.

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**Abstract no.: P09.30**

**Eradication Rate of *H. pylori* in Patients Coming from East Europe Compared to Italian Patients: Results of a Clinical Trial**

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**Aim:** To compare the efficacy of a standard triple therapy lasting 7 days administered in immigrant patients living in Italy and in
Italian patients to eradicate *Helicobacter pylori* infection, and to compare the peptic ulcer disease (PUD) prevalence between these patient groups.

**Methods:** One hundred and sixteen (62 female/54 male, median age: 39 years; 25th/75th: 29/48 years) consecutive *H. pylori*-infected immigrant patients living in Italy were recruited between 2008 and 2009, as were 112 (56 female/56 male, median age: 55 years; 25th/75th: 47/64 years) consecutive *H. pylori*-infected Italian patients. All underwent 13C-urea breath test (UBT) and endoscopy with biopsies and considered infected, if both tests were positive. All patients were offered a standard 7-day triple therapy (rabeprazole 20 mg, clarithromycin 500 mg, and amoxicillin 1, twice daily). Eradication was assessed 4–6 weeks after end of treatment using 13C-UBT.

**Results:** Demographics of 116 infected immigrants: 44.8% Romania, 26.7% Ukraine, 19.8% Moldavia, 7.7% Croatia, and 0.86% Poland. The two populations differed for median age (p < .01), prevalence of PUD [54.3% in immigrants vs 28% in Italians, difference of proportions: 25.7%; 95% confidence interval (CI): 13 to 37.3; p < .01] and smoking status [62% smokers (immigrants), 42% (Italians); difference: 20.1%; 95% CI: 7.1 to 32.2; p < .01]. Twelve Italians (11%) and 16 immigrants (14%) dropped the study (p = .479). The eradication rate according to the intention-to-treat analysis was 70% (95% CI: 61.5 to 78.2) for Italians and 48.3% (95% CI: 39.4 to 57.3) for immigrants (difference: 22.3%; 95% CI: 9.5 to 34; p < .01). According to the per-protocol analysis the eradication rate was 79% (95% CI: 70 to 85.8) for Italian and 56% (95% CI: 40.6 to 65.3) for immigrant (difference: 23%; 95% CI: 10.1 to 35; p < .01). A multivariate logistic regression analysis including country of origin, sex, age, PUD, smoking, and alcohol status found that immigrant patients had an adjusted odds ratio for not eradicating equal to 2.14 (95% CI: 1.13 to 4.06).

**Conclusions:** *H. pylori*-infected immigrant patients treated with the standard triple therapy seem to be more at risk of unsuccessful eradication, compared to Italian patients. Further studies are demanded to confirm and clarify these intriguing results.

**Abstract no.: P09.31**

**Effects of *H. pylori* Eradication on Serum Pepsinogen I, II, Ratio and Gastrin-17 in Italian and Immigrant Patients**

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**Aim:** To study the influence of *Helicobacter pylori* eradication on serum gastrin-17 (sG-17), pepsinogen I (sPGI), pepsinogen II (sPGII), and sPGI/sPGII in infected Italian and immigrant patients.

**Methods:** Fasting serum before and 2 months after end of eradication treatment was analysed for sG-17, sPGI, and sPGII using Gastropanel ELISA (Biohit) in 116 (62 female/54 male, median age: 39 years; 25th/75th: 29/48 years) consecutive *H. pylori*-infected immigrant patients and in 112 (56 female/56 male, median age: 55 years; 25th/75th: 47/64 years) consecutive *H. pylori*-infected Italian patients recruited 2008–2009. All underwent 13C-urea breath test (UBT) and endoscopy with biopsies, and considered infected, if both test were positive. All patients were offered a standard 7-day triple therapy (rabeprazole 20 mg, clarithromycin 500 mg, and amoxicillin 1, twice daily). Eradication was assessed 4–6 weeks after end of treatment using 13C-UBT.

**Results:** The eradication rate (ER) according to the intention-to-treat (ITT) analysis was 70% [95% confidence interval (CI): 61.5 to 78.2] for Italians and 48.3% (95% CI: 39.4 to 57.3) for immigrants (difference: 22.3%; 95% CI: 9.5 to 34; p < .01). According to the per-protocol (PP) analysis the ER was 79% (95% CI: 70 to 85.8) for Italians and 56% (95% CI: 40.6 to 65.3) for immigrants (difference: 23%; 95% CI: 10.1 to 35; p < .01). After 2 months, in eradicated patients there was a significant decrease of 13% (95% CI: 10 to 16) for sPGI, 56% (95% CI: 54 to 56) for sPGII, an increase of 99% (95% CI: 85 to 213) for sPGI/sPGII, and a decrease of 20% (95% CI: 16 to 25) for sG-17. There was no significant difference between eradicated patients of both populations. Among the noneradicated patients a slightly but significant increase in sPGI (6%; 95% CI: 3 to 9) was observed. Receiver-operating characteristic curves were plotted for sPGI, sPGII, and sG17 2 months after the eradication treatment as a predictor of *H. pylori* eradication. The area under the curve (AUC) for sPGII performed after 2 months was of 0.968 (95% CI: 0.955 to 0.997) providing a sensitivity of 98.3% (95% CI: 91 to 99.7) and a specificity of 90% (95% CI: 83.2 to 94.7) to predict the eradication using a cut-off ≥10.5 µg/L. No significant difference in AUC was found between immigrant and Italian patients (p = .77).

**Conclusions:** Eradication of *H. pylori* causes a significant decrease of sPGI, sG17, and in particular of sPGII. Serum pepsinogens and gastrin-17 can be a useful noninvasive tool to follow up the functional state of the gastric mucosa after treatment and sPGII a potential useful marker to follow up *H. pylori* infection.

**Abstract no.: P09.32**

**Eradication of *H. pylori* for the Prevention of Peptic Ulcer Rebleeding: A Long-Term Follow-up Study of 700 Patients**


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**Aim:** To evaluate the effect of *Helicobacter pylori* eradication on ulcer bleeding recurrence in a prospective, long-term study including 700 patients.
Abstract no.: P09.33
Histopathologic Detection of Helicobacter-like Organisms in Thoroughbred Horses with High Doses of Phenylbutazone

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Phenylbutazone is used as a non-steroidal anti-inflammatory drug (NSAID) for the treatment of chronic pain, including the symptoms of arthritis. In Venezuela phenylbutazone is permitted in the racecourse of Thoroughbred horses. Overdose or prolonged use can cause gastrointestinal ulcers, blood dyscrasia, kidney damage, oral lesions, and internal hemorrhage, especially pronounced in young, ill, or stressed horses. Helicobacter species have been detected and associated with equine gastric ulcer syndrome. The aim of this study was the histopathologic detection of Helicobacter-like organisms (HLO) in Thoroughbred horses treated with high doses of phenylbutazone. Equine were treated for 4 days with phenylbutazone at an intravenous dose of 5.5 mg/kg. All cases were examined by necropsy, histopathology, and special staining (Giemsa, Toluidine Blue, and Warthin–Starry). Samples of gastric mucosa were collected from 64 Thoroughbreds in the National Race Track “La Rinconada” Caracas, Venezuela. Macroscopic pathology showed surface gastritis acute (15 of 64), chronic gastritis with focal erosion (27 of 64), chronic gastritis with ulcer (22 of 64), and (25 of 64) with chronic collits. Histopathologic lesions were surface gastritis acute in 15 of 64, chronic gastritis with ulcer in 22 of 64, and (25 of 64) with chronic collits. Special staining showed spiral-shaped HLOs: Giemsa (+) (47/64), Toluidine Blue (+) (49/64), and Warthin–Starry (+) (51/64). Seventy-eight percent (78%) of Helicobacter spp. (HLO)-infected equine treated with high doses of phenylbutazone had severe gastritis lesions. These results suggest that Helicobacter species and concurrent use of phenylbutazone predispose to a high damage in the gastric mucosa in Thoroughbreds.
C in 2, and D in 1. All nine subjects (8.9%) with RE and esophageal ulcer were negative for H. pylori infection. Gastric ulcer was detected in 12 subjects (6 H. pylori positive, 6 negative), and duodenal ulcer in four (1 H. pylori positive, 3 negative). The incidence of gastroduodenal ulcer was 15.8% (16 of 101). Subjects were surveyed using the gastrointestinal symptom rating scale (GSRS), with no differences in scores for acid reflux, abdominal pain, or indigestion according to the presence or absence of RE, peptic ulcer. The significant correlations with RE were hiatal hernia and H. pylori infection negative. No significant correlations were seen for peptic ulcer.

**Conclusions:** Upper gastrointestinal mucosal injuries (ulcer and mucosal break) associated with LDA therapy were found not only in the stomach, but also the esophagus and duodenum.

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**P10 Molecular Genetics and Genomics, Virulence Factors and Pathogenesis II**

**Abstract no.: P10.01**

**Development of Adsorption Method to Study H. pylori Proteins Expressed In Vivo During Infection**

A. Santhanam,* R. Noordin, ′ L. Chun Wei, ′ S. Sreenivasan† and N. Velusamy†

*Institute for Research In Molecular Medicine, Pulau Pinang, Malaysia; ′Hospital Sebarang Jaya, Pinang, Malaysia

The study of in vivo induced antigen helps to identify immumogenic Helicobacter pylori proteins expressed specifically during infection and this requires the use of well-adsorbed serum samples. H. pylori was isolated from biopsies obtained from patients referred to esophagogastroduodenoscopy at Seberang Jaya Hospital, Penang, Malaysia. The isolate was subjected to cultivation, gram staining, a series of biochemical tests, and polymerase chain reaction (PCR) to confirm the isolate as H. pylori. A suitable growth media to support the growth of H. pylori ATCC 700824 and the clinical isolates were evaluated. Brain heart infusion broth was suitable for liquid culture, while TSA with 5% defbrinated sheep blood was the solid media to support the growth of H. pylori. The serum samples obtained from healthy volunteers and patients (suspected H. pylori infection) were screened to confirm the status of H. pylori infection through standard techniques of sodium-dodecyl-sulphate polyacrylamide gel electrophoresis (SDS PAGE) and Western blotting. A method was successfully developed that adsorbed the in vitro antibodies from the sera of both positive and negative H. pylori infection by using whole cells antigen of H. pylori cultured in *in vitro*, followed by evaluation using ELISA.

**Abstract no.: P10.02**

**Genotyping of H. pylori in Archival Gastric Biopsies**


Adelaide and Meath Hospital, Tallaght, Ireland

**Introduction:** Genotyping alterations of Helicobacter pylori are thought to be responsible for the various clinical manifestations in the host. There are two phenotypically distinct H. pylori groups: type one H. pylori, which express cagA and vacA, and type two where cagA is absent and vaculating cytoxin activity is not manifested although vacA gene is present. Type one are more strongly pathogenic than type two.

**Aims:**

1. To compare the genotype profiles of H. pylori from archived gastric tissue in patients with chronic gastritis (CG) and intestinal metaplasia (IM).

2. To evaluate a nested polymerase chain reaction (PCR) assay for diagnosing and identification of virulent H. pylori in archived gastric biopsies and their main virulence genes cagA, vacA, and iceA.

**Methods:** Eighty-seven patients were divided into two groups, IM group and CG group.

- DNA extraction and nested PCR were performed on archived cut sections from consecutive paraffin-embedded histology blocks of gastric biopsies.

- The H. pylori DNA integrity and specificity were confirmed by the ureC PCR.

**Result:** - Amplification of the cagA gene was present in 43% of isolates, and was more frequent in (IM) patients than of (CG) patients (68%, 17%).

- The vacA combination s2m2 genotypes were the most common allelic combinations of the vacA gene.

- The iceA1 gene was more prevalent than iceA2, 38% and 20%, respectively. Five percent of the isolates were positive for both iceA1 and iceA2 and 20% were negative for both. Prevalence of cagA, vacA1m1, and iceA1 was significantly associated with IM patients (p = .001, p = .002, p = .001, respectively).

**Abstract no.: P10.03**

**Immobilization of a Specific Receptor for H. pylori Using Model Surfaces**

P. Parreira, † C. A. Reis, ‡ A. Magalhães, ‡ D. Leckband‡ and M. C. L. Martins†

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A new approach to prevent and eliminate Helicobacter pylori from infected hosts, based on nanostructured biomaterials with capacity to attract and bind the bacteria is our main goal. For that,
C in 2, and D in 1. All nine subjects (8.9%) with RE and esophageal ulcer were negative for *H. pylori* infection. Gastric ulcer was detected in 12 subjects (6 *H. pylori* positive, 6 negative), and duodenal ulcer in four (1 *H. pylori* positive, 3 negative). The incidence of gastroduodenal ulcer was 15.8% (16 of 101). Subjects were surveyed using the gastrointestinal symptom rating scale (GSRS), with no differences in scores for acid reflux, abdominal pain, or indigestion according to the presence or absence of RE, peptic ulcer. The significant correlations with RE were hiatal hernia and *H. pylori* infection negative. No significant correlations were seen for peptic ulcer.

**Conclusions:** Upper gastrointestinal mucosal injuries (ulcer and mucosal break) associated with LDA therapy were found not only in the stomach, but also the esophagus and duodenum.

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**P10 Molecular Genetics and Genomics, Virulence Factors and Pathogenesis II**

**Abstract no.:** P10.01  
**Development of Adsorption Method to Study *H. pylori* Proteins Expressed In Vivo During Infection**

**A. Santhanam,** R. Noordin, L. Chun Wei, S. Sreenivasan† and N. Velusamy†  
†Institute for Research In Molecular Medicine, Pulau Pinang, Malaysia; †Hospital Sebarang Jaya, Penang, Malaysia

The study of in vivo induced antigen helps to identify immunogenic *Helicobacter pylori* proteins expressed specifically during infection and this requires the use of well-adsorbed serum samples. *H. pylori* was isolated from biopsies obtained from patients referred to esophagogastroduodenoscopy at Seberang Jaya Hospital, Penang, Malaysia. The isolate was isolated to cultivation, gram staining, a series of biochemical tests, and polymerase chain reaction (PCR) to confirm the isolate as *H. pylori*. A suitable growth media to support the growth of *H. pylori* ATCC 700824 and the clinical isolates were evaluated. Brain heart infusion broth was suitable for liquid culture, while TSA with 5% defibrinated sheep blood was the solid media to support the growth of *H. pylori*. The serum samples obtained from healthy volunteers and patients (suspected *H. pylori* infection) were screened to confirm the status of *H. pylori* infection through standard techniques of sodium-dodecyl-sulphate polyacrylamide gel electrophoresis (SDS PAGE) and Western blotting. A method was successfully developed that adsorbed the in vitro antibodies from the sera of both positive and negative *H. pylori* infection by using whole cells antigen of *H. pylori* cultured in *vitro*, followed by evaluation using ELISA.

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**Introduction:** Genotyping alterations of *Helicobacter pylori* are thought to be responsible for the various clinical manifestations in the host. There are two phenotypically distinct *H. pylori* groups: type one *H. pylori*, which express cagA and vacA, and type two where cagA is absent and vaculating cytotoxin activity is not manifested although vacA gene is present. Type one are more strongly pathogenic than type two.

**Aims:** 1. To compare the genotype profiles of *H. pylori* from archived gastric tissue in patients with chronic gastritis (CG) and intestinal metaplasia (IM).  
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**Methods:** Eighty-seven patients were divided into two groups, IM group and CG group.  
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- The iceA1 gene was more prevalent than iceA2, 38% and 20%, respectively. Five percent of the isolates were positive for both iceA1 and iceA2 and 20% were negative for both. Prevalence of cagA, vacAs1m1, and iceA1 was significantly associated with IM patients (p = .001, p = .002, p = .001, respectively).

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glycosylated receptors (Gly-R) specific to H. pylori will be immobilized onto the biomaterial surface. In order to study the specificity of the interaction between H. pylori and immobilized Gly-R, these receptors must be immobilized onto surfaces that avoid nonspecific adhesion.

To identify our base surface, we first needed to understand the behavior of this bacterium in the presence of different surface chemistries. For that, we used model surfaces (self-assembled monolayers – SAMs) with different exposed functional groups: CH₃, OH, and tetraethylene glycol (EG4). SAMs are ideal models, since they are stable, easy to produce, easy to functionalize allowing a precise control of immobilized ligands. Three H. pylori strains were tested: Hp J99, Hp 17875/Leb, and Hp 17875babA1A2 mutant. Bacteria viability was also evaluated. Results demonstrated that H. pylori adhesion to EG4-SAMs is very low for at least 24 hours.

Therefore, Gly-R was immobilized to mixed SAMs containing EG4 and streptavidin bound to biotin-functionalized alkanethiols.

Mixed SAMs were characterized using X-ray photoelectron spectroscopy, water contact angle, and ellipsometry. Streptavidin and the subsequent biotin-Gly-R adsorption to biotinylated mixed SAMs were followed using quartz crystal microbalance with dissipation (QCM-D).

We acknowledge Prof. T. Borén for H. pylori strains.

Abstract no.: P10.04
Determinants of H. pylori Localization and IL8 Expression in the Human Gastric Mucosa

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The density of Helicobacter pylori on the gastric mucosa may be influenced by a number of factors. Duodenal ulcer promoting gene A (dupA) is associated with interleukin (IL)-8 secretion and duodenal ulcer (DU). Gastric cancer is associated with pan-gastritis, and DU with antral-predominant gastritis, but the determinants of distribution of inflammation in the stomach are unknown. We aimed to investigate the effect of H. pylori virulence factor expression on the gastric cytokine response to infection, and determine the impact of these on bacterial localization and colonization density in humans.

The host immune response was assessed in 20 H. pylori-positive patients by quantification of IL8 mRNA expression by real-time polymerase chain reaction (RT-PCR). Virulence genotypes of colonizing strains were determined by PCR for cagA and dupA. Bacterial colonization density was assayed by RT-PCR.

Colonization with a dupA+ strain was associated with a 5-fold increase in IL8 expression compared to dupA− infections (median 120 vs 23 units, p = .027). A 6-fold increase in antral-corpus bacterial colonization was observed with dupA+ (median = 2.8 units) compared to dupA− infections (median = 0.5 units, p = .025), suggesting that dupA+ strains have a tendency towards antral predominant colonization. A negative association between IL8 expression and bacterial density was seen.

Infection with a dupA+ strain was associated with a more aggressive inflammatory response and antral-predominant colonization, consistent with the increased risk of duodenal ulceration attributed to this virulence factor. Bacterial colonization density appeared to follow a reciprocal association with inflammatory cytokine expression implying that inflammation may limit H. pylori growth in the human gastric mucosa.

Abstract no.: P10.05
The Development of Selective Inhibitors of H. pylori Gamma-glutamyltranspeptidase

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All Helicobacter pylori-infected patients develop chronic gastritis, which may proceed to gastric cancer. Despite a strong local and humoral immune response, the bacterium is not eliminated. In earlier studies, we have identified the gamma-glutamyltranspeptidase (gGT), which is secreted by H. pylori as a central inhibitor of the human immune response in the stomach by inhibiting lymphocyte proliferation.

Such effect towards lymphocytes is not described for gamma-glutamyltranspeptidases from any other species. We have therefore expressed and purified gGTs from different species in order to compare the substrate specificities and elucidate the unique functional properties of the H. pylori gGT by establishing structure-functional relationships. Enzymes displayed big differences in substrate-specific activities as determined by kinetic measurements. Furthermore, recombinant gGT enzymes from species other than H. pylori displayed no significant inhibitory effect on T-cell proliferation compared with HpgGT.

We further extended our search for novel inhibitors of H. pylori gGT by comparative in silico screening and identified putative compounds with an inhibitory effect on H. pylori gGT activity. Relating the binding affinity and potency of these compounds towards gGTs of different species with their predicted structural and mechanistic models enables us to develop species-specific, selective gGT inhibitors.

Abstract no.: P10.06
Adhesion of H. pylori to Gastric Epithelial Cells Influenced In vitro by L-Dopa: An Explanation for Clinical Differences in Bioavailability?

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Recent investigations on the pharmacokinetics of levodopa (L-Dopa) indicated that the presence of Helicobacter pylori in Parkinson disease patients, orally treated with L-Dopa, influences the absorption of this compound and leads to decreased plasma levels. Therefore this work aims to study a potential in vitro interaction of L-Dopa with H. pylori and its surface adhesins. To investigate the influence of L-Dopa on the bacterial adhesion a
flow cytometric assay with was developed. Therefore fluorescein isothiocyanate (FITC)-labeled bacteria were preincubated with the tested substance, followed by incubation with gastric epithelial cells (AGS) and quantitative flow cytometric analysis. In addition free L-Dopa was quantified from the incubation supernatants with *H. pylori* by high-pressure liquid chromatography with ultraviolet detector. Quantitative evaluation of time and concentration-dependent incubation experiments indicated a significant decrease of L-Dopa concentrations when getting in contact with *H. pylori*. The reduction of L-Dopa concentrations was determined with 47% to 12% referred to the initial starting concentration, with time-dependency and dependency of the *H. pylori* density. FITC-labeled *H. pylori*, preincubated with differing L-Dopa concentrations, was shown to have a significant (p < .05) reduced bacterial adhesion to AGS cells with maximum reduction of 22±19%. Binding experiments of FITC-labeled *H. pylori* to human gastric tissue sections confirm the adhesion reduction induced by L-Dopa. These results demonstrate a direct interaction of L-Dopa with outer membrane proteins of *H. pylori*, responsible for the adhesion to gastric epithelial cells. This study suggests a potential in vitro interaction of L-Dopa with *H. pylori* adhesins, confirming the clinical changes found in pharmacokinetics of L-Dopa therapy by *H. pylori*-positive Parkinson patients.

**Abstract no.: P10.07**

**Protein Differential Expression According to the *H. pylori*-Associated Pathology**

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Differences among *Helicobacter pylori* strains do certainly contribute to the fact that only a subset of individuals develops an infection-associated gastric pathology, although half of the world’s population is infected. Highlighting these differences, we made a large spectrum comparative proteome analysis among 13 different strains clinically isolated from colonized patients but still with normal mucosa and those with epigastric pain, gastritis, peptic ulcer, and gastric cancer. After protein separation by two-dimensional gel electrophoresis and by using ImageMaster™ 2D-Platinum software we detected 17 protein spots differentially expressed according to the *H. pylori*-associated pathology. Differences were considered statistically significant for p values < .05. Our results indicate that 41% (7 of 17), 12% (2 of 17), and 6% (1 of 17) of these protein spots were underepressed in normal, ulcer, and epigastric cancer clinical isolates, respectively. On the other hand, 24% (4 of 17) and 18% (3 of 17) were overexpressed among gastric cancer and normal clinical isolates, respectively. All these proteins are being identified by protein mass fingerprinting using MALDI-TOF mass spectrometer.

Although the proteome is quite similar among all the *H. pylori* strains included in the study, we were able to establish a correlation between the expression of specific proteins and the virulence of the bacteria. The identification of these proteins by mass spectrometry will contribute to elucidate the mechanisms underlining virulence, becoming valuable for prognosis, diagnosis, and therapeutics of *H. pylori* infections.

**Abstract no.: P10.08**

**Detailed Sequence Analyses for the C-Terminal Region of CagA Protein of *H. pylori***

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The pathogenic CagA protein of *Helicobacter pylori* contains a highly polymorphic Glu-Pro-Ile-Tyr-Ala (EPIYA) repeat region in the C-terminal. The segments contain EPIYA motifs which have been designated as segments A, B, C, and D. This study used 560 unique CagA sequences containing 1796 EPIYA motifs collected from public resources, including 274 Western and 286 East Asian strains with clinical data obtained from 433 entries. Fifteen types of EPIYA or EPIYA-like sequences are defined. In addition to four previously reported major segment types, six minor segment types, EPIYA-B′, -C′, -D′, -B″, -C″, and -D″ were defined using our classification method. In total, 41 different sequence types (e.g., ABC, ABD) were identified. By pattern comparisons, we found that the EPIYA motif belongs to motif type EPIYA-C if the EPIYA sequence is immediately followed by TIDD, TIEE, TIDE, SIDD, TIDD, TIAE, or TIAD; and belongs to motif type EPIYA-D if it is followed by TIDE or TIDS. We confirm that the sequences from Western and East Asian strains contain segment C and D, respectively. All Western strains contain segment C; however, approximately 8% of East Asian strains contain segment C, indicating that there was partial transmission of *H. pylori* from Western to East Asian countries. Finally, we also confirm that strains with two EPIYA segment C have a greater chance of developing gastric cancer than those with one segment C. However, phylogenetic analysis did not reveal any association between a particular disease and a specific CagA sequence.

**Abstract no.: P10.09**

**Genotyping of *H. pylori* in Bile Samples from Patients with Cholecystitis**

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**Aim:** Despite of several recent reports on the detection of *Helicobacter pylori* DNA in human bile, there are still uncertainties concerning the correlation of these findings with biliary tract and liver diseases. The aim of the present study was the detection of *H. pylori* in bile samples and to evaluate the incidence of bacteria genotypes (*babA2*, *cagA*, *vacA* s1/s2, and *m1/m2*) in patients with cholecystitis.

**Material and Methods:** Using polymerase chain reaction (PCR), we detected the presence of *H. pylori* in bile samples from 61 persons, of which 42 patients had chronic noncalculous cholecystitis (17 male/25 female, mean age is 45 years) and 19 patients had

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calculus cholecystitis (7 male/12 female, mean age is 60 years). Thirty of 91 patients, comprising the control group, had no biliary tract diseases. The detection of H. pylori DNA and the presence of bacterial virulence genes were performed by PCR using specific primers for H. pylori ureC as well as by cagA, vacA/s1A2 PCR according to the manufacturer’s recommendation (“Lytech”, Russia).

Results: H. pylori was found in 21 (50%) bile samples of patients with chronic noncalculus cholecystitis and in only two (10.5%) of the 19 samples of patients with calculus cholecystitis. In the control group none of the samples showed the presence of H. pylori. The cagA and babA2 were identified in nine (39.1%) and five (21.7%) cases, respectively. The frequencies of cagA and babA2 genotypes in patients with cholecystitis was vacA/s1/m2* cagA−babA2*.

Abstract no.: P10.10
Functional Analysis of CagA Polymorphism in Iranian H. pylori Strains

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Introduction: It seems that cagA polymorphisms are due to homologous recombination occurred within the 3′ region of the gene. This variety of recombination results in highly heterogeneous EPIYA repeat region of CagA among various Helicobacter pylori strains. In this study, we assessed the CagA polymorphism in terms of hummingbird phenotype.

Methods: A total of 110 single colony strains recovered from various dyspeptic patients were studied. Polymerase chain reaction (PCR) amplification of cagA 3′ variable region using cag2F and cag4R primers was followed by sequencing. H. pylori strains were cocultured with AGS cells for 24 to 48 hours prior to their examination for the hummingbird phenotype. The percentage of cells with hummingbird characteristics was calculated through image processing. The statistical analysis of data was performed using Kruskal–Wallis test.

Results: Results revealed that 88% of the studied strains possessed the variable region. Sequencing of cagA amplics with various sizes provided different subtypes as AB (8.2%), BC (4.1%), ABC (53.6%), ABCC (29.9%), and ABBBCC (4.1%). We found that hummingbird phenotype formation was significantly prominent in AGS cells incubated with strains having ABCC type than all other divergent subtypes (p < .05).

Conclusion: This study demonstrated that our strains with various CagA subtypes display notable differences in the level of hummingbird phenotype induction. Such phenotype variation may explain important differences in the pathogenicity of H. pylori strains. Collectively, we found a functional link between CagA polymorphism and hummingbird phenotype which enables us to determine the risk of H. pylori-associated diseases in our populations where the cagA prevalence is uniformly high.

Abstract no.: P10.11
H. pylori Virulence Determinants and Their Association with Ethnicity and Disease in a Multi-ethnic Country

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Introduction: Helicobacter pylori-related disease development is attributable, at least in part, to the genotype of the infecting strain. While several virulence determinants have been linked to disease development, associations have been inconsistent between populations. This study examined 12 virulence determinants in isolates from three ethnic groups resident in Malaysia and Singapore. These ethnic groups have disparate H. pylori prevalences and gastric cancer (GC) incidence rates.

Methods: H. pylori isolates were cultured from 159 symptomatic patients resident in Malaysia and Singapore [52 Chinese, 49 Indian, and 20 Malay functional dyspepsia (FD), 16 Chinese duodenal ulcer (DU) and 22 Chinese gastric cancer (GC)]. Polymerase chain reaction (PCR) was used to detect dupA, cagA, cagE, cagT, cagL, and babA, and to type the cagA EPIYA motifs, the vacA signal sequence, midregion and intermediate regions, and the HP0521 allele/restricted deletion. The on/off status of oipA was determined by sequencing.

Results: The prevalence of cagA, cagE, cagL, cagT, babA, oipA ON, vacA s1, and vacA i1 was >85% in isolates irrespective of ethnicity or disease state of the host. The predominant HP0521 allele, EPIYA motif, and dupA prevalence varied significantly with ethnicity (p < .05).

Conclusion: The majority of isolates assessed carried the H. pylori virulence investigated, including an intact cag PAI. The novel association between HP0521 alleles and host ethnicity is of specific interest. In contrast to previous studies no association was found between specific virulence factors and disease state. The multivariate analysis currently being undertaken may reveal additional associations between combinations of virulence determinants and disease state.

Abstract no.: P10.12
Association of H. pylori dupA and oipA Genotypes with Clinical Outcome in Irish Patients

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Introduction: Helicobacter pylori infection leads to ongoing functional damage to gastric mucosa and is a major cause of gastric
malignancy. Some bacterial strains express virulence factors (dupA and oipA) that increase the risk of clinical disease outcomes. Numerous studies have reported worldwide variations in genotyping of H. pylori and the incidence of gastroduodenal diseases.

**Aims:** The aim of this study was to investigate the relationship between the presence of dupA and oipA genes in H. pylori strains isolated from Irish adult patients with gastroduodenal diseases.

**Methods:** DNA was extracted from 140 H. pylori strains obtained from patients with intestinal metaplasia (IM), chronic gastritis (CG), and peptic ulcer (PU) group patients. The dupA genes were amplified by using the primers JHP917 and JHP918.

**Results:** Overall both jhp0917 and jhp0918 (42%). The presence of dupA was high in CG (50%), compared with those from PU (35%) or IM (42%). The oipA gene was amplified in all H. pylori isolates, oipA “on” status was closely related to cagA, vacAs1a, and babA2 genes.

**Conclusion:** Based on the polymerase chain reaction method the carriage of oipA “on”/vacAs1a genotypes in the presence or absence of cagA may increase the risk for the development of peptic ulcer and precancerous lesions. The presence of dupA is not an ideal marker for duodenal ulcer in an Irish population.

**Abstract no.: P10.13**

**Number and Sequences of CagA EPIYA Motifs in H. pylori Strains Isolated from Patients Descending from Indigenous Chilean Population are Conserved**

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H. pylori infects almost half of the world’s population with colonization rates in Chile reaching 73%. This bacterium is associated with late-developing gastric cancer, a sequelae more common in an indigenous Chilean people, the Mapuches, compared to the rest of the country’s population. CagA, a virulence factor involved in the bacterium’s pathogenesis, is transferred to epithelial cells by a type IV secretion system, phosphorylated at EPIYA motifs by Src-type host kinases. Phosphorylated CagA interferes with intracellular signaling pathways, altering cellular processes such as cell proliferation, apoptosis, immune response, as well as cell morphology in general. Previous CagA sequence analyses have revealed A, B, C, and D EPIYA motifs. Strains with higher virulence are theorized to carry a higher number of EPIYA motifs (up to 5), with D (Eastern type) and C (Western type) associated with more aggressive strains. However, recent studies in Korean, Malaysian, and Costa Rican populations question this theory. Using polymerase chain reaction amplification and cagA partial sequencing, the number and type of motifs in strains isolated from a group of infected indigenous Chilean people, the Mapuches, were analyzed. The number and type of EPIYA motifs were conserved. Moreover, cagA genes with only three EPIYA elements in the A-B-C arrangement, containing only two Western-type CagA multimerization motifs, were identified. Since these features do not represent the most aggressive EPIYA arrangement, these findings do not explain the high gastric cancer incidence reported for this population compared to infected non-Mapuche Chileans. Funded by grants CTU06 Biomedicina Area 5 and Fondecyt 1085232.

**Abstract no.: P10.14**

**A Triple Positive H. pylori Strains (CagA, VacA S1, and BabA2) are Associated with Childhood Duodenal Ulcer in Russia**

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**Background:** Some years ago Gerhard et al. (1999) showed that the combined genotype of BabA2, CagA, and VacA S1 (“triple-positive” strains) demonstrated significant correlation to the presence of duodenal ulcer (DU) in European adult population.

**Aim:** The aim of this study was to determine the association of triple-positive (CagA/VacAS1/BabA2) strains (TPS) of Helicobacter pylori with peptic ulceration in children living in Russia.

**Methods:** H. pylori strains were isolated from gastric mucosa of 39 pediatric patients with DU (26 males, mean age 12.6 ± 2.4 years) and 73 pediatric patients with chronic gastritis (CG) without DU (34 males, mean age 12.06 ± 3.1 years).

Genomic DNA of H. pylori was extracted from all clinical isolates. Polymerase chain reaction technique was used to characterize the presence of CagA, VacA S1, and BabA2 genes in the isolates.

**Results:** TPS were detected in 61.5% patients with DU and only in 24.6% patients with CG without DU. TPS showed significant association with the presence of DU (p = .00014). When the simultaneous presence of CagA, VacA S1, and BabA2 was studied, we found that 75.8% of isolates with VacA S1 genotype were BabA2-positive, as were 84% isolates with CagA genotype.

**Conclusion:** We completely agree with Gerhard et al. (1999) opinion that the TPS in patients with CG should be eradicated, because the presence of combination of CagA, VacA S1, and BabA2 may lead to DU.

**Abstract no.: P10.15**

**H. pylori and Host Factors Involved in Gastric Cancer Development**

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**Introduction:** Helicobacter pylori is assumed as the causative agent in developing gastric adenocarcinoma. H. pylori high prevalence...
and high gastric cancer (GC) mortality in Iran have promoted molecular and serologic studies on *H. pylori* virulence markers as well as host potential risk factors in GC progression. This study aims to explore associations between *H. pylori* markers, pepsinogen II/III, and proinflammatory cytokines and their associations with risk of GC.

**Methods:** Blood samples were obtained from 225 healthy volunteers, 641 patients who underwent gastrointestinal endoscopy, and 310 GC patients. Histopathology was done by an expert pathologist. PGI, PGII, and *H. pylori* status were analyzed by ELISA. IL-1B and IL-1RN were genotyped by specific polymerase chain reactions.

**Results:** In dyspeptic and GC population, 83.8% and 87.2% were *H. pylori* seropositive, respectively. Among non-GC-infected population, 69.5% and 88.5% were VacA and CagA seropositive. In GC-infected cases, 78.4% and 97.1% were VacA and CagA seropositive. Antibodies against *H. pylori* 35 and 37 kDa proteins were significantly protective against gastric GC (p < .05). Antral and corpus atrophic gastritis were not associated with seroreactivity toward *H. pylori* antigens (p > .05). In contrast, atrophic body gastritis was associated with PGI/PGII < 2.5 (p < .05). *H. pylori* seropositivity and the presence of VacA antibodies showed statistically significant associations with lower PGI/II ratios. No statistical difference was observed between *H. pylori* seroreactivity and IL-1 alterations, while these associations were associated with GC compared to healthy controls (odds ratio 1.3; p < .05).

**Conclusion:** In this study, *H. pylori* was not the only factor involved in stomach carcinogenesis. Human genetic content and environmental risk factors should be considered as potential risk factors in GC development.

### Abstract no.: P10.16
**A New Approach for Rapid Isolation and Purification of Alkyl Hydroperoxide Reductase from *H. pylori***

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Alkyl hydroperoxide reductase (AhpC) of *Helicobacter pylori* is known as the virulence factor because of its activity to *H. pylori* survival against oxidative environment of gastric mucosal layer. This conserved antigen has been described as a specific and unique enzyme for *H. pylori* and therefore, both *H. pylori* AhpC and Anti-AhpC could be useful in the development of serologic and stool antigen tests, to detect and monitor *H. pylori* infection. In this study, a new convenient approach has been used to purifying it.

The isolation and purification of AhpC from *H. pylori* were attempted by various techniques including ammonium sulfate precipitation, dialysis, preparative sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and electroelution and in any step enzymatic activity of AhpC was determined. AhpC was purified 100-fold with an overall recovery of 60% from clinical isolates of *H. pylori*.

This approach is simple and time- and cost-saving for purification of AhpC enzyme from *H. pylori*.

### Abstract no.: P10.17
**H. pylori Genotypes in Pediatric Patients with Celiac Disease**

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**Background:** A high diversity of *Helicobacter pylori* strains and their genomic variability may influence clinical outcome in humans. Celiac disease is frequently associated with chronic gastritis which is caused mainly by *H. pylori*.

**Aim:** The objective of present study was to determine the prevalence of selected virulence-associated *H. pylori* genes: cagA, vacA (alleles s1/s2, m1/m2), iceA, babA, and dupA in clinical isolates obtained from patients with celiac disease.

**Materials and Methods:** The study was performed on *H. pylori* strains (n = 30) collected in years 2006–2008. The strains were isolated from gastric biopsies of children, aged 2–18 years, diagnosed and treated for celiac disease. The genes encoding virulence factors, such as: cagA, vacA (s1/s2, m1/m2), alleles iceA1, iceA2, babA, and dupA were detected by multiplex polymerase chain reaction. Antibodies against *H. pylori* antigens: VacA (95 kDa) and CagA (120 kDa) were detected in patients’ sera by Western blot.

**Results:** In examined *H. pylori* strains s1/m1 vacA, cagA+ genotype was present in 11 of 30 strains (36.66%), whereas s1/m2 vacA, cagA+, and s2/m2 vacA, cagA– genotypes in eight of 30 (26.66%), respectively. Genes encoding virulence factors: iceA1, iceA2, babA, and dupA were detected in 53%, 23%, 13%, and 43% of strains, respectively. The anti-CagA and anti-VacA *H. pylori* antibodies were detected in 69.23% patients.

**Conclusions:** The high prevalence of anti-CagA and anti-VacA antibodies in correlation to cagA+ vacA+, iceA1 *H. pylori* genotype demonstrates the necessity for routine diagnosis of *H. pylori* infection in patients with celiac disease. The *H. pylori* genotype may have influence on clinicopathologic features of celiac disease.

### Abstract no.: P10.18
**CagA and VacA genotype Prevalence in *H. pylori* Isolated from Madrid, Spain**

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Prevalence of vacA and cagA genotypes of *Helicobacter pylori* in Spain is unknown. The aim of this study was to determine CagA and VacA genotypes among *H. pylori* isolated in Madrid, Spain.
We obtained 118 *H. pylori* strains from biopsies of patients with gastric symptoms, from June 2008 to January 2009. DNA extraction was carried out by the NucliSens easyMAG platform (BioMérieux). *VacA* genotypes (s, m) were determined by polymerase chain reaction (PCR) and agarose gel. *CagA* status was determined by PCR. Within the *CagA* negative, we confirmed the absence of the pathogenicity island by “empty-site” PCR. The identification of the number and type of *CagA* EPIYA motifs was based on sequencing analyses.

The results of *vacA* and *cagA* are shown in the Table 1. Spanish patients were more often colonized with *cagA*-negative strains than patients born outside of Spain (*p* < .0001). *CagA* with three EPIYA motifs (ABC) was observed in 63.3% of 44 *cagA*-positive strains. Strains with more than three EPIYA motifs were more often recovered from adults than from children (*p* < .003). We found the EPIYA-ABD in patients from non-East Asian countries.

In Madrid, Spain, most of *H. pylori* isolates are *vacA* s2/m2 and *cagA* negative. The majority of the *cagA*-positive strains have three EPIYA motifs.

**Table 1** VacA and *cagA* genotypes in strains isolated in Madrid

<table>
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<th><em>cagA</em> status</th>
<th>s1/m1 or m2</th>
<th>s2/m2</th>
<th>s2m1 and mix strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative (n = 74)</td>
<td>3 (4.1%)</td>
<td>69 (93.2%)</td>
<td>2 (2.7%)</td>
</tr>
<tr>
<td>Positive (n = 44)</td>
<td>35 (79.6%)</td>
<td>3 (6.8%)</td>
<td>6 (13.6%)</td>
</tr>
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